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JOURNAL OF DAIRY SCIENCE

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PROGENY TEST AND INDIVIDUAL PERFORMANCE AS INDICATORS OF AN ANIMAL'S BREEDING VALUE

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It has long been known by breeders that sometimes a few offspring indicate an animal's real breeding value more accurately than the animal's own appearance or performance does. The rediscovery of Mendelism and the discovery a little later (by Bateson and others) of complementary genes and of other epistatic relations among non-allelomorphic genes, provided a simple explanation for this. Occasionally there are cases where a single offspring furnishes more convincing proof of its parent's genotype than could ever be obtained by studying the parent itself. So far as color is concerned, this would be the case with a purebred red calf born in one of the black breeds of cattle.

These are special cases, deserving attention to be sure, but perhaps they are more significant as *exceptions* to a general rule than as *examples* of one? Some writers on animal breeding topics have considered them to be examples. For instance we are sometimes told that a progeny test is "genotypic" and therefore dependable, whereas a cow's own record is "phenotypic" and therefore not dependable. But no fundamental distinction is really involved, since the progeny test itself consists of some kind of an average of "phenotypic" records of the offspring. To suppose that the process of averaging would purge these phenotypic records of all their error and would bring out only the pure gold of genotypic truth seems to require too much faith in the potency of arithmetic!

The present paper presents the results of an inquiry into the biometrical relations which determine the relative accuracy of progeny test and parent's own performance as indicators of the parent's real breeding value. The complete answer contains many variables but most of these have little influence. An answer accurate enough for practical purposes can be presented in usably simple form. For convenience the problem is phrased in terms of dairy cattle breeding thus: Is a cow's own record or the average record

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of n of her daughters the more dependable indicator of the cow's own breeding value?

METHODS USED AND DEFINITIONS

These biometrical relations were explored by Wright's method of path coefficients (10). Figure 1 shows those relations. The small letters indicate path coefficients which measure (provided the cause and effect relationships are portrayed correctly in the diagram) the importance of the causes of variation in the effect. The path coefficient (h) from heredity (H) to observed characteristic or yield (C) is the ratio of the standard deviation of C due to H to the total standard deviation of C. The path coefficients h and e may be used to express the relative importance of heredity and environment on production records in a particular population of data. Because variation is measured by squares of deviations h and e will not add up to unity. Where there is no correlation between heredity and environment, the squared path coefficients, h^2 and e^2, will come near to expressing "the relative importance of heredity and environment" in the form in which that idea is ordinarily encountered in practical problems. A definite numerical value for h^2 is a description of the population from which it was derived and may not, without other knowledge, be safely applied to populations where the variations in environment or in heredity may be quite different. For prediction purposes, path coefficients are regression coefficients expressed on a scale in which each standard deviation is one unit.

Most of the terms in Figure 1 are self-explanatory. Phenotype is used here to mean what the outwardly observed record would be if it were possible to keep all environmental circumstances rigidly controlled. It differs from the actually observed record in that the latter has been modified by the variations in environment to which the various phenotypes were exposed. Non-additive effects of environment on the phenotype (such as concern Hogben so much in his recent "Nature and Nurture" (5)) may be minimized by expressing the records on scales which show in the most nearly additive way the effects of variations in environment and in heredity. For example there is some evidence to indicate (but perhaps not enough to prove conclusively?) that dairy production records should be expressed in logarithms of the yield instead of in pounds if the most efficient use is to be made of the information in those records. However, for the present question, non-additive combination effects of phenotype and environment may be considered as included in the environment, since they tend to decrease the correlation between records and genotypes.

"Genotype" is obtained by assigning each gene such a value that the simple sum of all the genes in each individual will give the "genotype" for that individual subject to the "least squares rule," that (1) the average

genotypic value of the population shall coincide with its average phenotypic value and (2) the sum of the squares of the individual deviations of "genotype" from "phenotype" shall be a minimum.* This "genotype" seems to be the same as Fisher's "expected value" of which he says (4, page 32), using human stature for illustration: "This expected value will not necessarily represent the real stature, though it may be a good approximation of it, but its statistical properties will be more intimately involved in the inheritance of real stature than the properties of that variate itself."

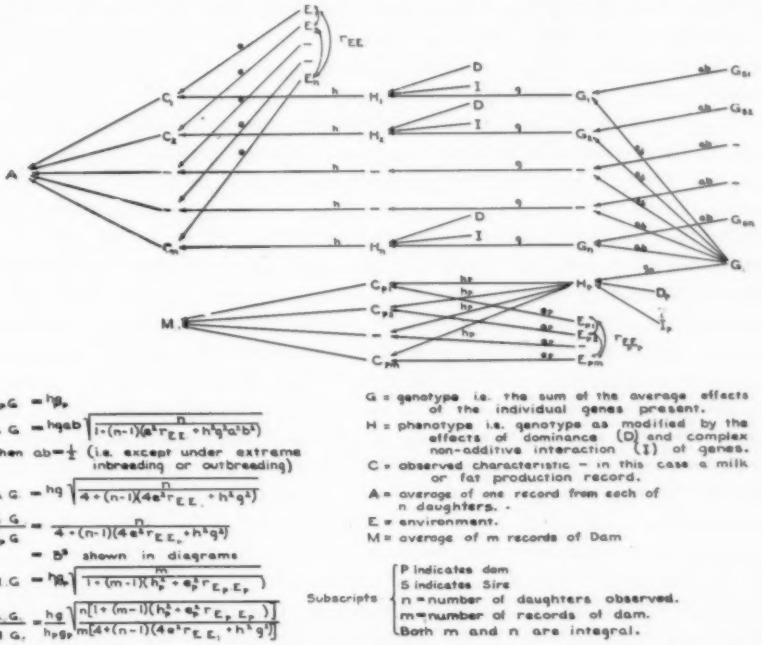
A
M
D
I
G = genotype i.e. the sum of the average effects of the individual genes present.
H = phenotype i.e. genotype as modified by the effects of dominance (D) and complex non-additive interaction (I) of genes.
C = observed characteristic – in this case a milk or fat production record.
A = average of one record from each of n daughters.
E = environment.
M = average of m records of Dam
Subscripts
P indicates dam
S indicates Sire
n = number of daughters observed.
m = number of records of dam.
Both m and n are integral.
When ab = ½ (i.e. except under extreme inbreeding or outbreeding)

FIG. 1. PATH COEFFICIENT DIAGRAM SHOWING BIOMETRIC RELATIONS BETWEEN PARENT'S RECORDS AND PARENT'S BREEDING VALUE (G) AND THE BIOMETRIC RELATIONS BETWEEN THE PROGENY AVERAGE AND THE PARENT'S BREEDING VALUE.

These genotypic values will be perfectly correlated with Turner's definition (9, page 45) of "transmitting abilities," in so far as dominance is the only cause of discrepancy between genotype and phenotype, but Turner's definition makes no provision for non-additive combination effects of genes which are not allelomorphic to each other. For convenience in this article such non-additive combination effects of non-allelomorphic genes will be

* I am indebted to Dr. Sewall Wright for this method of expressing as much as possible of hereditary differences in terms which contribute to the correlations between relatives.

called epistasis or epistatic effects, although this is a slightly broader meaning than that for which "epistatic" was first used in genetics. In the usage of practical animal breeders, "nicking" most nearly has the same meaning but many breeders also apply the term, "nicking," to phenomena which are nothing but unusual Mendelian recombinations of genes each of which may have nearly additive effects.

"Genotype" as used here is perfectly correlated with breeding value if the latter term is understood to express the average merit of the offspring produced by mating this animal to mates which are a truly representative sample of their breed. If the mates are not a truly representative sample and if there is much epistatic interaction or if there is much dominance and mating is far from random, it must sometimes happen that an individual will produce offspring of higher (or lower) merit in one kind of herd than it would in other herds where the most frequent gene-combinations are quite different. In such cases an animal's breeding value as measured by actual records of progeny, will deviate from what its breeding value would be if its mates were truly representative of their whole breed. However such offspring will not transmit such dominance deviations or epistatic deviations, since those deviations are functions of the *gene combination* rather than of the individual genes considered separately. The *combination* is not transmitted but segregates at the reduction division and the genes reassemble in other combinations in the next generation. Hence "genotype" as used here to express the sum of the average effects of the constituent genes *is* "breeding value" if one considers mating the animal to representative animals of its breed, though it may deviate from "breeding value" if the latter term is used to mean the contribution which this animal could make to descendants produced by breeding it to some isolated and non-typical sub-group of the breed.

"Phenotype" is used here as a convenient intermediate step to show the modification of the genotype by dominance or epistatic relations, which are usually considered genetic phenomena although really they are only consequences of the chemistry and physiology by which the genes produce their effects and are not involved in the mechanism by which genes are transmitted. By this device we can express separately the proportion (h^2) of the observed variance which is hereditary in the broader sense of that word (*i.e.*, including the effects of dominance and epistasis as "hereditary") and the proportion (g^2) of the hereditary (in that broad sense) variance which can be expressed by this additive scheme for representing genotype. The proportion of the observed variance which is hereditary in the narrow sense of that word (*i.e.*, which would contribute directly to parent-offspring correlations) would be h^2g^2.

Non-additive effects of gene combinations are separated into those due to dominance (D) and all others (I). This is done merely because the sta-

tistical consequences of dominance have been explored (2, 3, 12) and can be stated in definite formulae. The statistical consequences of epistatic interactions are not so well known and must be quite complex in some cases, but on the whole probably are much the same as those of dominance, *i.e.*, to lower the correlation between genotype and outward characteristic and hence to lower the observed correlations between relatives, but to affect the correlations between some kinds of relatives more than those between other kinds. So far as dominance and epistasis merely lower the parent-offspring correlation (*i.e.*, diminish the value of g) their effects nearly cancel out of the present problem since they weaken the correlation between daughter average and dam's genotype almost as much as they do the correlation between the dam's average and her genotype. However so far as dominance and epistasis lower the correlation between sisters less than they do that between parent and offspring, they actually prevent the progeny test from becoming relatively as accurate an indicator of the dam's genotype as it would if there were no dominance or epistasis.

The importance of epistasis in practical breeding problems is still a matter of conjecture, with some workers thinking it very unimportant and others speculating that it may often be highly important, especially in herds already improved to a point considerably above the average level of the breed. Wherever the parent-offspring correlation is high under nearly random mating (after having been corrected for the effects of common environment), it seems likely that epistasis is unimportant. However a low parent-offspring correlation under those circumstances does not prove that epistasis is important, since such a low correlation might also result from h being small, *i.e.*, from random variations in environment having important effects on the records.

The importance of dominance depends primarily on whether complete dominance is the rule, how abundant the dominant genes are in contrast to their recessive allelomorphs, and somewhat on the deviations from random mating. In a population mating at random with dominance complete and with no epistasis, g^2 for the effects of each gene pair $= \frac{2(1-q)}{2-q}$ or conversely $d^2 = \frac{q}{2-q}$ = the portion of variance in H due to dominance. (The letter q here represents the frequency of the dominant genes among all the genes occupying that locus in the whole breed.) This d^2 which Fisher calls "the dominance ratio" (3) is thought by him to be typically about $\frac{1}{3}$ for most characteristics. Wright (12, pages 137–139) for other reasons, which appear to him more convincing, believes that this ratio is more typically about $\frac{1}{5}$, even if complete dominance is the almost universal rule among all genes. Moreover he questions (13) whether such complete dominance is the rule, especially among modifying genes each of which has

a comparatively small effect. Such modifying genes probably furnish much of the variance in quantitative characteristics which, like milk production, are vitally necessary in some degree but which may vary widely among different individuals in the breed.

Whether complete dominance is the rule or not, it is obvious that dominance will not often be the major obstacle in estimating an animal's genotype from its own appearance or performance. Even if dominance is universal and Fisher's estimate of d^2 is correct, g will generally have a value of about .82, while if Wright's view is correct g will usually be nearer .90. Both these figures are based on the assumption that epistasis is too small to be important. If epistasis is important, d^2 and g^2 would still retain about the same relation to each other, though both would be smaller. There will be occasional characteristics (of which lethals are notable examples) where dominance is complete and the recessive gene is so rare that the "dominance ratio" approaches close to unity. In those cases dominance may be the major source of the discrepancies between genotype and phenotype. However in such traits the recessive individuals are rare and each such trait will contribute but little to the total variance of the population. For example, in the United States in black breeds of cattle like the Angus, Galloway, or Holstein-Friesian, the gene for black must be around ten to fifteen times as frequent as its recessive allelomorph. Hence the "dominance ratio" for this particular trait will be well above .80. But the gene for red is so rare that less than 1% of the purebred calves of these breeds are born red. Hence this trait is a comparatively unimportant source of loss to breeders. Dominance has been discussed here more than its importance justifies, largely because it has received more attention than it deserves in the writings on applied genetics.

SOLUTION UNDER SIMPLEST CONDITIONS

Figure 1 shows the relations and correlations as they would be under random mating. The correlation between one record of the dam and the dam's genotype will be $h_p g_p$ while the correlation between one record of one daughter and the dam's genotype will be hgab. When m unselected records of the dam are averaged together the correlation between that average and the dam's genotype becomes

$$h_p g_p \sqrt{\frac{m}{1+(m-1)(h_p^2+e_p^2 r_{E_p E_p})}}$$

which approaches

$$g_p \sqrt{\frac{h_p^2}{h_p^2+e_p^2 r_{E_p E_p}}}$$

as a limit as m becomes indefinitely large. If $e_p^2 r_{E_p E_p} = \text{zero}$ (the most favorable condition for high values of r_{MG}), this approaches $r_{MG} = g_p$ as a

limit. Increasing the number of records observed thus tends to eliminate errors arising from random uncorrected variations in environment but does not tend to eliminate errors arising from dominance or epistasis or consistently biased and uncorrected environment.

On the other hand, the average record of n daughters each tested once is correlated with the dam's genotype thus:

$$hgab\sqrt{\frac{n}{1+(n-1)(h^2g^2a^2b^2+e^2r_{EE})}}$$

which approaches

$$\sqrt{\frac{h^2g^2a^2b^2}{h^2g^2a^2b^2+e^2r_{EE}}}$$

as n becomes indefinitely large. This approaches unity when e^2r_{EE} is zero. In other words, increasing n tends to cancel errors arising from the imperfections of both h and g, whereas increasing m tends to cancel the imperfections of h_p but does nothing to correct for the fact that g_p is less than unity. However the progeny test involves ab which arises from the intervening generation of Mendelian segregation and appears not at all in the correlation between dam's record and dam's genotype.

Thus a daughter's record usually is a less accurate (on account of ab) indicator of her dam's genotype than the dam's own record is, but the errors in daughters' records tend to be more completely cancelled by the averaging process than is the case with errors in the dam's record. Hence if g and g_p were quite small, it *might* be possible for n to be so large that its cancellation of errors arising from dominance or epistasis would more than make up for the Mendelian sampling errors which affect the progeny average but not the dam's own records.

The problem of the relative accuracy of progeny test and dam's own record as indicators of the dam's genotype therefore becomes the quantitative one of balancing the errors introduced by ab against the greater effectiveness of the progeny average in eliminating errors introduced by dominance and epistasis. Unfortunately for the simplicity of the answer, e^2r_{EE} and $e_p{}^2r_{E_pE_p}$ cannot safely be considered zero and their existence sets serious limits on the effectiveness of averaging as a means of eliminating errors, either from the daughter average or from the average of the dam's own records.

The value of ab will be nearly .5 even with moderately large departures from random mating (11, pages 118–119). Substituting that value for ab, the ratio (B) of r_{AG} to r_{MG} becomes

$$B=\frac{hg}{h_pg_p}\sqrt{\frac{n}{m}}\sqrt{\frac{1+(m-1)(h_p{}^2+e_p{}^2r_{E_pE_p})}{4+(n-1)(h^2g^2+4e^2r_{EE})}} \qquad (1)$$

If dam and daughters are produced by the same kind of a breeding system $g = g_p$ and if dams and daughters are equally typical samples of their generations and if the records used to represent the productiveness of dam and of daughters are chosen by a method equally fair to both, $h = h_p$ and the first part of this expression cancels. Assuming (as the case most favorable to the progeny test) that $m = 1$ and squaring to simplify the expression we have

$$B^2 = \frac{n}{4 + (n-1)(h^2g^2 + 4e^2r_{EE})} \qquad (2)$$

For the progeny test to be more accurate than the dam's own record B^2 must be larger than 1.0. This condition is satisfied when $n > 4 + (n-1)(h^2g^2 + 4e^2r_{EE})$. For this to be true, n must be larger than 4, even when h^2g^2 and e^2r_{EE} are extremely small. If e^2r_{EE} is as large as .25, n cannot possibly be large enough to make the progeny test average as reliable as the dam's own performance.

Equation 2 is pictured graphically in figures 2 and 3. Since four variables are involved (treating h^2g^2 as a single variable and e^2r_{EE} as another single variable, since each behaves as such in this statement of the problem) it seems necessary to select certain reasonable or limiting values of one of the four and for this constant value of one variable to show the interrelations of the other three as a curved surface whose height above a base is B^2.

Figure 2 is designed to show how B^2 varies with n and with h^2g^2. The upper surface shows the limiting condition (e^2r_{EE} = zero) most favorable to the progeny test. Such a condition would be encountered in actual practice when half sisters resemble each other only because they have the same dam. Any correlation between half sisters because they are kept under similar environment, or are related through their other parent, or because r_{DD} or r_{II} are not zero would have the same kind of effect as giving e^2r_{EE} a positive value. Some such effects are almost certain to occur in any set of actual data, even when such data have been obtained under experimental conditions controlled as rigidly as possible. The lower surface in figure 2 shows B^2 when $e^2r_{EE} = .20$. Such a condition would arise whenever unrelated cows in the same herd showed a correlation of +.20 just because they were managed and tested in the same herd in a population of data coming from many herds kept under varying management. Such a value for e^2r_{EE} is roughly consistent with much of the C.T.A. and Official data yet analyzed in a way which permits any estimate of e^2r_{EE}. However the value of e^2r_{EE} naturally will vary from population to population, since it is a description of the heterogeneity of the conditions under which different sets of half sisters are kept.

The double line in figure 2 shows a plane parallel to the base at the level $B^2 = 1.0$. Points above this level denote conditions under which the progeny test is more accurate than the dam's own record. When $e^2r_{EE} =$ zero, and h^2g^2 is as low as .10, the progeny test surpasses the cow's own record when $n = 5$, but n must exceed 6 when $h^2g^2 = .4$ and must exceed 11 when $h^2g^2 = .7$. This brings out the important points that the progeny test

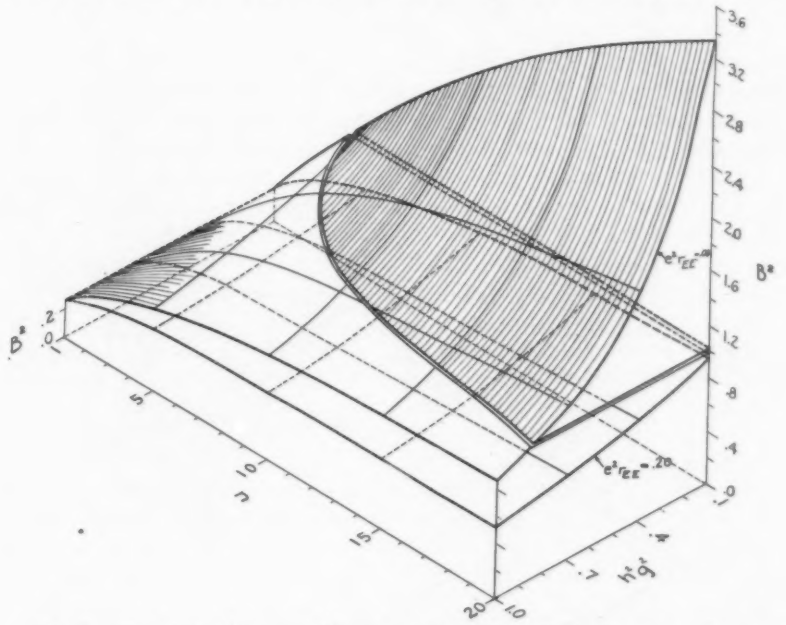

FIG. 2. The relative accuracy of progeny test and the individual's own performance when the number (n) of offspring in the progeny test varies and for traits which are highly hereditary (h^2g^2 is large) or slightly hereditary (h^2g^2 is small). The upper surface shows this condition when the offspring resemble each other only because they are half-sibs. The surface at $e^2r_{EE} = .20$ shows the condition when the offspring resemble each other enough for other reasons to bring about a correlation of +.20 between them on that account alone.

is not very dependable where the daughters resemble each other for other reasons than that they are out of the same dam[1] and that the superiority of the progeny test over the dam's own record is most marked where h^2g^2 is smallest, *i.e.*, where the characteristic is least hereditary in the narrow sense

[1] This same point was shown in more detail by the present writer in an article (8) directed primarily at how r_{AG} varies with increasing n. In that article e^2 was used to include all the causes for half sisters resembling each other except that they are by the same parent.

of that word, and therefore where neither indicator is highly accurate! Values of h^2g^2 less than .10 are not shown in either figure 2 or figure 3. For such values B^2 would become very large if e^2r_{EE} were also very small.

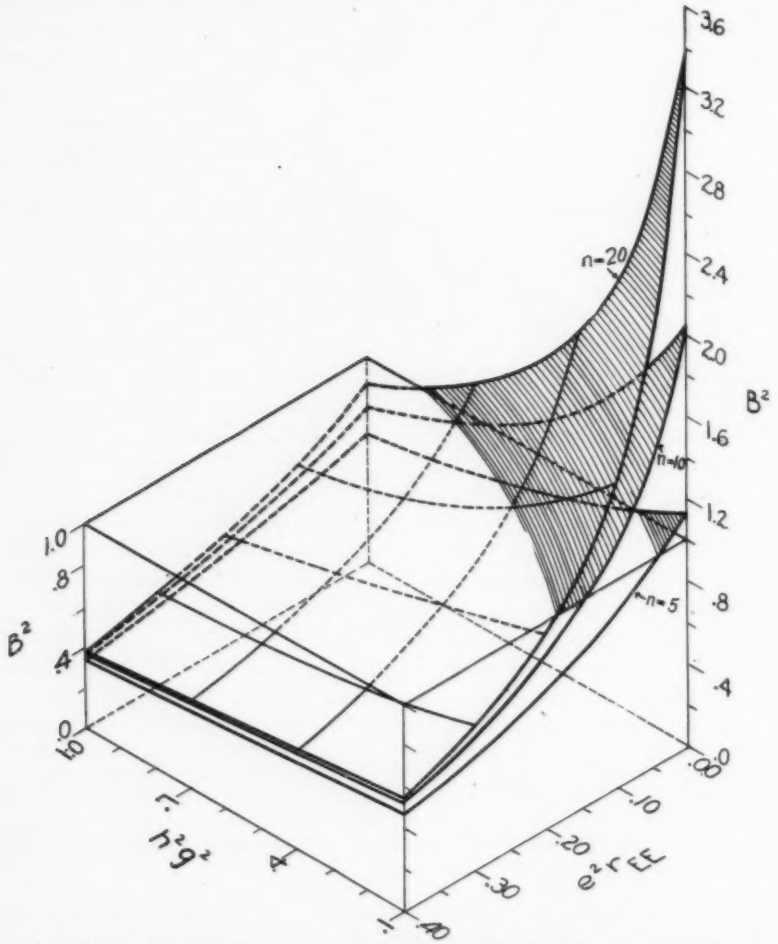

FIG. 3. The relative accuracy of progeny test and individual's own performance for three selected values of n (20, 10 and 5) for traits in which the importance of heredity varies and for populations in which the other reasons for offspring resembling each other vary from zero to a correlation of +.40 between the offspring for other reasons than that they are out of the same dam.

Figure 3 shows how h^2g^2 and e^2r_{EE} affect B^2 for three selected values of n (5, 10, and 20). The shading shows the regions where B^2 rises above 1.0.

The limiting effect of e^2r_{EE} on the accuracy of the progeny test is vividly shown in this figure. Only rarely can the accuracy of the progeny test in a whole population average as high as that of the parent's own record, if the correlation between herd mates due to herd environment is as high as .10 and if h^2g^2 is as large as .10.

CONDITIONS WHICH MAY AFFECT THE VALIDITY OF THE SIMPLE SOLUTION

Figures 2 and 3 show the situation when m is one, each daughter is represented by one record only, breeding is at random, the sires are of unknown breeding value, dams and daughters are produced by the same kind of breeding system, and dams are just as representative of their generation as daughters are of theirs. Now these conditions will never be fulfilled exactly, not even in experimental data. It is important to glance for a moment at the way deviations from these conditions will affect figures 2 and 3, although only deviations from the last condition can alter those figures materially in the direction of making the progeny test more reliable.

When m is more than one—and it usually will be if n is as large as two—the dam's record will be more reliable, in accordance with the formula in equation (1). When each daughter is represented by an average of several records, the effect is that of increasing h and making the progeny test more reliable but this is usually more than offset by the increase in m. Any dam who has several daughters, each with more than one record, is almost certain to have had many records of her own, so that the average number of records per daughter will nearly always be less than m. Hence the use of an average of all available records will tend to make both indicators more reliable but will usually increase that of the dam more than that of the progeny.

Deviations from random mating may be of two kinds so far as they affect this problem. First there may be a correlation between the mates of a cow, irrespective of whether they are like or unlike her. A positive correlation of this kind has the same effect as an increase in e^2r_{EE} and therefore lowers the reliability of the progeny test. A negative correlation of this kind (*i.e.*, some degree of success in deliberately seeing to it that each cow was mated both to very good bulls and to an equal number of very poor bulls) would increase the accuracy of the progeny test slightly since it would eliminate some of the chance existing under truly random breeding, that one cow would happen to be mated only to good bulls, another only to poor bulls, etc. However such a negative correlation cannot be high if n is large, because if a cow is mated to three or more bulls those bulls cannot each one be exactly opposite to every other one! Moreover it is hard to imagine that such a general negative correlation could exist under any circumstances except a breeding experiment where it was made

a cardinal point to get from each cow daughters by poor bulls and daughters by good bulls.

The second kind of deviation from random mating would be a general correlation between dams and sires so that it would generally be true through the whole breed that good cows were usually mated to good bulls, poor cows to poor bulls, etc. This could come about either through inbreeding or through deliberate assortative mating based on individual performance or the performance of near relatives. It is not unreasonable, either from the genetic principles involved or from the published studies of dairy data to suppose that this correlation within dairy breeds might well be as large as +.15 or +.20. So long as all breeders are working for high production, this correlation could hardly rise much above +.25 or +.30 without the practice of extreme inbreeding. The effect of such a general resemblance between sire and dam would be to increase the accuracy of the progeny test slightly, since each offspring would then tend to be like the dam, not only because of receiving half its inheritance from her but also because the other half of its inheritance came from a sire whose inheritance was more like that of the dam than if he had been chosen at random from all sires of the breed. A general correlation of +.25 between the genotypes of sires and dams would modify equation 2 enough that the progeny test might possibly be more accurate than the dam's own record while n is as small as 3, but this would be true only if e^2r_{EE} were zero and h^2g^2 were very small, and there were no other correlation between sires. Since it is not often that all three of these conditions will be fulfilled, it seems unlikely that such deviations from random mating as exist in actual dairy cattle populations will often alter figures 2 and 3 enough to make the progeny test in a whole population average as accurate as the cow's own record when the cow has as few as four daughters. It should be mentioned that in plants where self-fertilization is possible, the resultant perfect correlation between genotypes of sire and dam is quite attainable and makes it possible for a progeny test based on as few as two offspring to be as accurate as the parent's own record. This is doubtless the most important fundamental reason that plant breeders make so much use of the progeny test, particularly when working with self-fertilized organisms like wheat.

Sometimes the merits of the different sires will be partly known. If their genotypes were perfectly known and there were no correlation between sires and dams, formula 2 would become:

$$B^2 = \frac{n}{4 + (n-2)h^2g^2 + 4(n-1)e^2r_{EE}} \qquad (3)$$

Thus even such extreme knowledge as this would have but a trifling effect on figures 2 and 3. If mating is not at random, such information about the sires is quite useful in that it would cancel and prevent errors which would

otherwise be introduced by any such correlation between sires as that discussed in the next to the last paragraph. Knowledge of the sires therefore prevents a correlation between sires from causing the accuracy of the progeny test to fall far below the levels pictured in figures 2 and 3, but cannot of itself raise those levels much higher than is shown. If there is a general correlation between sires and dams and the merit of the sires is known from other evidence, then such knowledge will help to predict the dam's genotype but such help is because of that direct correlation between the genotypes of sire and dam, and not because of evidence from the progeny themselves.

Where dam and daughters have been produced by different breeding systems, the values of g for dam and daughter may not be identical and hence will not cancel out of equation 1 as has been assumed so far. The most extreme example of this, so far as concerns dominance, would be the case where the progeny test is conducted by breeding the cows to homozygous recessive bulls. In such a case the deceiving effects of dominance would become zero for the daughters and a correspondingly higher percentage of the total variance among them than among the dams would be truly genetic in the narrow sense of that word. Naturally such a test could not be made for a cow on account of her short life and limited reproductiveness, but a corresponding test of a bull might be practical if the information gained were worth the cost of getting it. However dominance is usually only a minor cause for actual records deviating from breeding value. The assembling and maintaining of a herd of recessive cows for such tests would be expensive, even if possible at all. Moreover such a plan does not appear to offer any help for avoiding deception by epistatic effects. These considerations make it seem quite unlikely that such a testing plan would often yield information worth the cost of getting it. If the sire and dam belonged to different breeds, there would be increased probability of important misleading epistatic effects which would probably preclude using for testers individuals of a breed developed for other purposes, as for example the use of beef-bred cows for testing the breeding value of a dairy bull for milk and fat production.

Some of the most brilliant research in genetics, particularly in the genetics of Drosophila and of maize, owes its success to the devising and use of tester strains so designed as to eliminate (except for Mendelian sampling errors) the effects not only of dominance but also of epistatic interactions, thus making the truly genetic portion of the variance (h^2g^2) among the offspring much higher than was the case among their parents. However the devising of such a tester strain has for its prerequisite a reasonably accurate hypothesis as to the number and kind of genes involved and (at least so far as concerns epistasis) of the precise way in which the effect of the gene combination differs from the sum of the average separate effects

of the genes concerned. The limited reproductive rate of farm animals, their comparatively long interval between generations and their expensiveness make it well nigh a lifetime's work to create such a tester strain, even if the investigator were wise enough or lucky enough to have chosen the correct working hypothesis at his first attempt and even if the genetic situation were simple. Hence it appears unlikely that much use can be made of such tester strains in the breeding of farm animals, however useful and even necessary such tester strains may be in research on fundamental principles of genetics.

The assumption that daughter and dam are equally representative of their generation and that their records were selected by methods equally fair to both will often be untrue especially in semi-commercial data, and is the only one of these assumptions likely ever to be important enough in a whole population to make much change in figures 2 and 3. The effect of such differences between daughters and dams is to make h in figure 1 unequal to h_p. If they are unequal these two terms cannot be cancelled out of equation 1 as has been inferred hitherto.

If but one record is used to represent each animal and if that record is selected on some such *ex post facto* basis as the choice of the highest record after having seen them all, such a selection deliberately picks out that record made under the most favorable combination of unknown conditions of environment and health. Such selection automatically tends to give to the cow having many lactations a figure less truly representative of her phenotypic producing ability than would be the case with a cow which had but one or two lactations from among which to select. Since the dam usually has more records than the daughter, the net result is to make the dam's record usually less typical of her than the daughter's record is typical of her. This tends to make the progeny test more accurate than is pictured in figures 2 and 3. It should be pointed out that this comes not from any intrinsic superiority of the progeny test, but rather from the bias introduced by the choice of records where variable numbers are available. Studies of the actual correlations between records of the same cow in different lactations in the same herd have generally given values ranging from about + .30 to + .60. Correlations as low as this give ample room for selections of the largest record for each cow to cause such selected records to deviate far from each cow's phenotype, especially if some cows have only one or two records and others have 4 or 5, from among which to select.

Probably more generally important is the fact that the dams are often a more highly selected group than the daughters are. Usually all of the progeny which were tested at all are included in the progeny test. If they were very poor producers, they may have been culled shortly afterward but their records remain for use in the progeny test. The conditions may be quite otherwise among the dams. Not all cows which are tested succeed

themselves in becoming dams of tested daughters. The dams being studied in any particular set of data are to some extent only the more favored survivors from among the group tested contemporarily with them. In studying correlations between the records of those dams and any measure of the subsequent performance of their progeny, one is by no means making a comparison between phenotypic and genotypic manifestations of production. Instead he is comparing the utility of those phenotypic differences still remaining among the dams after this selection has been practiced, with the utility of various averages of phenotypic differences among the less highly selected group of daughters. This seems to be a major part of the explanation for the practical findings of Jull (6, 7) and Copeland (1) that the ancestor's production record is of relatively limited value in breeding selections. In Jull's data all birds which laid less than 200 eggs were culled before they had a chance to become dams. What would have happened, if all hens or a strictly random sample of those hatched in each generation had been progeny-tested, of course can only be surmised. Copeland's data came from many herds and at various dates, so that selection was not by such uniform rules as in Jull's experimental data. Nevertheless Copeland's tables 1 and 5 show that there had been stronger selection among the dams than among either their daughters or granddaughters, else the extreme regression (about 50 to 70 pounds of fat) from the dams' average to the daughters' and granddaughters' averages would not have occurred.

Naturally such studies as those of Jull and Copeland (many others might be mentioned but these two are selected as typical of the more careful among recent ones) correspond closely to the conditions practical breeders actually face. Depending on the amount of selection involved and on how highly hereditary the trait really is, it may sometimes be true that such variation as remains among a highly selected group of dams may be less useful as an indication of the hereditary differences between those dams than the variation which exists among the relatively unselected daughters of those dams, even when there are as few as two daughters per dam. The point to be made here is that when this actually is the case, it is not because of any inherent biological superiority of the progeny test but rather it is because in the case of the dams we are comparing what *further selection* could do among a group of survivors on which considerable selection has already been practiced, with all which selection could do among a group of daughters where very little selection has yet been practiced. The practical utility of such comparisons is beyond argument for the breeder who must actually use, in some combination or other, some data from the more heavily selected population and some data from the less selected population. There is, however, some danger of confusion as to *the utility of the initial selections* in the populations from which the dams first come, if such studies are interpreted as meaning that an individual's own record is in-

herently less representative of its breeding value than the average records of as few as two or three of its offspring.

GENERAL CONSIDERATIONS AND DISCUSSION

The general conclusion to be drawn from all these considerations is that only under rare and unlikely combinations of conditions would a progeny test based on as few as four-daughters-average in an unselected population as accurate an indicator of a dam's breeding value as the dam's own performance. If the trait is highly hereditary, in the narrow sense of that word, or if the progeny resemble each other very much for reasons of having been exposed to a common environment, many more than four daughters may be required or it may even be quite impossible for the progeny test to average more accurate in a whole population than the dam's own performance, no matter how many daughters there are.

The superiority of the progeny test is greatest for traits which are least hereditary in the narrow sense of that word. For traits which are very faintly hereditary and for which the offspring do not resemble each other for reasons of having been under common environment, the progeny test can become several times as accurate as the dam's own record, but for that to be true the trait must be so slightly hereditary that neither indicator can be highly accurate!

The progeny test is needed most where one sex cannot express the trait, as in milk and fat production in dairy cattle, egg production in poultry, prolificacy in swine, etc. Since the biometrical relations between sire's genotype and progeny average are the same as those between dam's genotype and progeny average (except for sex-linked inheritance which must be a small part of the total inheritance in mammals) the foregoing considerations apply also to the progeny test of the sire. However, for traits which the sire cannot express himself, there is nothing but a pedigree estimate of the sire against which to compare the accuracy of a progeny test of him. Since a progeny test surpasses even the best pedigree estimate when there are more than three progeny (provided the offspring do not resemble each other very much for any other reason except that they are by the same sire), the progeny test in such cases is much more useful and more urgently needed for the sire than for the dam. However such progeny tests will rarely tell as much about the sire as the available information correctly used will tell about a dam of nearly equal age. Enthusiasm over some such slogan as the current one that "The next best thing to a proved sire is the son of a proved sire" should not cause us to forget that such a son is one generation of Mendelian segregation away from this sire and that half of his inheritance (a little more than half if the probably small amount of sex-linked inheritance in mammals is also considered) comes from his dam

whose breeding value may usually be estimated more closely than that of his proved sire, if the available information is fully and fairly used.

Although the progeny test will not often be *superior* to the dam's own record (where no differences of selection are involved), it will be noticed from equation 2 that $B = .5$ even when n and m are only one. Therefore even the most fragmentary progeny test is worth much as an indicator of the dam's genotype. Naturally, as in any other prediction, both indicators should be used where both are available. The principles of multiple correlation govern the amount of attention to be paid to each indicator where both are used. However the formulae for the relative amount of attention to be given to the two records are not only complex but also involve a term (which is usually indeterminable) for the degree to which the dam's record and her daughters' records resemble each other because of being made under common environment. The ratio between the standard regression coefficients will be in the same direction from unity as is the ratio between the primary correlations (B in the earlier algebraic discussion) but will be more extreme. Perhaps as good an approximate rule as any would be to give a daughter's production nearly half as much attention as the cow's own record where there is only one record for each, but to give between $\frac{2}{3}$ and $\frac{1}{3}$ as much attention to the daughter average as to the cow's own record where there are at least two or three offspring. This rule would be approximately correct if daughters and dams had been equally selected from their generations. Where the dams have already been highly selected, more importance than this should be given to the progeny test in making further selections but it does not seem possible to develop any simple general rule for this, since such a rule would include terms for the intensity of selection among dams and among daughters and also a term for the extent to which the trait is really hereditary.

In actual practice selection will also be based in part on pedigree. Chronologically pedigree becomes available first, then the individual's own performance and the progeny test comes latest. As some selection is practiced on each basis, the possibilities of further gains by additional selections on the same basis rapidly diminish unless new information becomes available. The sampling nature of Mendelian inheritance sets a lower limit on the usefulness of pedigree than is inherently necessary for either of the other two bases of selection. Hence the very early selections on the basis of pedigree come near exhausting the possibilities in that direction, although increased knowledge of the performance of parents and other ancestors or collateral relatives does make it profitable occasionally to revise an earlier pedigree estimate. On the other hand, knowledge of the individual's own performance can continue to increase at a practically important rate as long as it lives and knowledge of its progeny can increase

as long as previously untested progeny come on test and (at a slower rate) as long as progeny already tested continue to be tested at later ages.

This leads to the general picture that pedigree occupies first place only for such selections as must be made before the other two criteria are available. It is very distinctly in third place after a few such early selections have been made. Individual performance occupies first place after it becomes available and until considerable use has been made of it. Then progeny test is the most useful basis for further selections but when it is much used for such selections, the possibilities in that tend to be exhausted and the individual's own performance might again assume first place. Among mature animals under practical conditions individual performance and progeny test therefore will vary or alternate as most useful for further selections, according to the amount of new knowledge becoming available about each and according to how nearly the possibilities for selection on the basis of the existing knowledge about each have already been exhausted.

SUMMARY

1. The biometrical relations governing the relative accuracy of progeny test and of the parent's own performance as indicators of the parent's breeding value are presented and discussed.

2. A solution under the simplest conditions is presented algebraically and graphically. Under those conditions there must be at least five offspring before the progeny test in a whole population will usually be a more accurate indicator of the parent's breeding value than the parent's own performance.

3. Most deviations from those simplest conditions have only slight effects on the solution. However any general resemblance between the offspring for any other reason than that they are half-sibs through the parent in question sets serious limits on the accuracy of the progeny test. On the other hand if the parents or the records used to represent them are more highly selected than the offspring or their records, the progeny test may become relatively more accurate than under the simple conditions for which the algebraic solution is given.

4. The progeny test is needed most for traits which cannot be expressed in one sex and for traits which are but slightly hereditary.

5. The bases for estimating breeding value are pedigree, own performance, and progeny test. As fast as some selection is practiced on one of these bases, the possibilities for further progress by additional selection on the same basis rapidly diminish and correspondingly increased attention should be given to one of the other bases.

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ELECTROKINETICS IN RELATION TO DAIRY PHENOMENA. I. THEORY AND METHOD

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For a long time it has been known that a number of dairy phenomena depend upon the coalescence or non-coalescence of the fat globules. Such dairy problems as the creaming of milk, the viscosity of cream and tendency to form cream plugs, the tendency of bottled cream to show a skim milk layer and the whipping ability of ice cream mixes are at present definitely known to involve the clustering of fat globules.

In the past a considerable amount of experimental work has been done on the practical factors that influence the above mentioned phenomena. Such studies, while of great value and importance, do not reveal the fundamental factor or factors that are operative.

Within recent years, it has been suggested by several investigators that the electrical effects at the fat-skim milk interface may be the governing factor, or at least one of the important factors in the behavior of milk fat globules under various conditions. With this suggestion in mind the present investigation was carried on.

I. HISTORICAL

Up to the present time, the literature concerning the electrical potential at the surface of the fat globule of cow's milk is very meager. Various references refer to such a charge, but most citations have been made purely on a speculative basis. However, a few references based on experimental work are available in the literature.

Sirks (28) in 1924 made a few observations on the electric charge of the fat globules. He concluded, from his preliminary work that the charge on the fat globule was of a low order of magnitude, and he was unable to correlate the charge with any dairy phenomena.

Mommsen (22) in 1929, using cataphoretic means, investigated the charge on the surface of the fat globule of cow's and woman's milk. He concluded that the fat globules of milk are normally negatively charged, in that they migrate in the direction of the anode when placed in an electrical field. Two experimental methods were used: first, the migration in "U" tubes was noted, and second, the pH was determined at which the iso-electric point was established in buffer mixtures.

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He found that the normal iso-electric point of the fat globule for cream diluted 1:100 was established at a pH of 4.17. While calcium ions added in the form of calcium chloride caused the iso-electric point to be raised. The addition of Ringer's solution to the buffer mixture caused the iso-electric point to be shifted to the acid side of pH 4.17. Additions of gelatin caused a shift to the iso-electric point of gelatin. This shift, he points out, was more evident with increasing concentrations of gelatin. The iso-electric point of the fat globules of human milk was found to be on the acid side of pH 4.17. This agrees with the recent work of Nugent (23) who found the iso-electric point of pH 4.1.

Prieger (27) has used cataphoretic means to study the nature of the hull surrounding the fat globule; her results, as in the case of Mommsen, are expressed in terms of the pH of the iso-electric point. She points out that the iso-electric point of artificial protein-free fat emulsions is more on the acid than is the case with the natural fat globules in milk. Because of this difference of hydrogen-ion concentration necessary to establish the iso-electric point she concludes that the natural fat globules of milk are surrounded by protein-like films, while the protein free artificial fat emulsion displays the true charge of the fat globule.

That the fat globules are surrounded by a layer of protein material, is in agreement with the findings of Titus, Sommer, and Hart (31) who arrived at the above conclusions by determining the nitrogen distribution of the fat "hulls" that were obtained by washing the fat globules. Hattori (15) (16) is of the opinion that the composition of the fat "hulls" is different from any of the other known proteins. Palmer and Wiese (32) have also investigated the "hull" material and conclude that it is composed of a mixture of protein and phospholipides.

Mohr and Brochmann (21), also using cataphoretic methods, have recently investigated the charge on the fat globule. These workers found a relationship between the churning ability of the cream and the charge on the fat globules. They did not, however, find any appreciable effect of such treatments as heating or homogenization on the pH of the iso-electric point. Neither did the addition of emulsifiers have any effect. They found that the iso-electric point of pure butter fat emulsions was more on the acid side than that of the fat emulsion in normal milk.

II. THEORY

The fact that colloidal systems, when placed in an electrical field, show the phenomenon known as cataphoresis was first firmly established by Linder and Picton (19) in 1892. Hardy in 1900 (13) noted that a suspension of egg albumin was positively charged (migrated to the cathode) in acid solution; but when placed in an alkaline solution it was negatively charged. In both cases the suspensions were stable, but when no migration

took place flocculation resulted. The same phenomenon was later observed by Burton (2).

As a result of cataphoresis experiments, Hardy (13) expressed the opinion that the presence of an electrical charge on the surface of a particle was an important factor governing its stability, since like charges on the particles would tend to keep the particles apart because of their mutual repulsion, and at the iso-electric point flocculation would occur. Powis (26) later pointed out that it was not necessary to bring the charge down to zero but only to a certain low order of magnitude (which he calls the critical potential) in order to bring about flocculation.

From such investigations as these, it has become quite generally accepted that the electrical charge is an important factor in the stability of colloidal systems.

However, it should be pointed out that in the case of one type of colloids known as emulsoids, there is an additional factor that must be considered. This factor is called by some the hydration of the particle. In order for flocculation of such a system to take place, it is necessary that both the electrical charge and the hydration factors be overcome or removed. In the case of emulsions a third factor, that of the emulsifying agent, must also be considered.

The original conception of Helmholtz (17) that the electrical charges existed in the form of a double layer, has been modified by Gouy (9) in that the charges constitute an ionic atmosphere. Such an "atmosphere" is not fixed but movable; the charged particle is free to move under the influence of an applied electromotive force. In cataphoresis we are concerned with the movement of particles suspended in a liquid under the influence of an applied potential. Streaming potentials refer to the E.M.F. set up by the movement of a liquid past a wall. Both types of electrokinetic phenomena are mathematically related.

Perrin (25), Briggs and Gortner (1) have derived the following expression of the streaming potential:

$$\zeta = \frac{HK_s 4\pi N}{PD}$$

ζ = zeta potential
H = observed E.M.F.
K_s = specific conductivity
N = viscosity
P = applied pressure
D = dielectric constant

It has recently been argued by Delbye and Hückel (3) that for a spherical particle the factor in the above equation should be six instead of four.

Kruyt (18) and Freundlich (7) have reported results of experimental work on the streaming of liquids through capillary glass tubes. The liquid was forced through under a measured hydrostatic pressure and the electro-

motive force that was set up was measured by suitable means. Their findings show that the observed electromotive force was independent of the cross section area or length of the tube. For a given liquid the E.M.F. that was set up was proportional to the applied pressure. However, they found that this value varied with the kind of glass that was used, but that the observed potential was constant for all shapes and sizes of tubes that were prepared from the same kind of glass.

Later Gee and Harrison (8) and Harrison (14) used the streaming potential method in developing their electrical theory of dyeing. They determined the potential of silk, wool and cotton against solutions of various electrolytes and dyestuffs. Fibers were packed in a cell between platinum electrodes and the E.M.F. that was set up when the liquid was forced through this diaphragm was measured.

Their findings show that acids increased the charge of positively charged fibers and decreased it on negatively charged fiber. All acids had the same effect, when the hydrogen-ion concentration was the same. Bases acted exactly opposite to that of acids. When an electrolyte containing polyvalent ions of opposite sign to that of the fiber was added, the charge was lowered, while in some cases it was even possible to reverse the sign.

Recently Briggs and Gortner (1) have used the streaming potential method for determining the potential existing at the cellulose-water interface. Gortner and his students (11) (12) have used the method extensively in studying the properties of the interface, including molecular configuration. Their method consists of forcing water, under a measured hydrostatic pressure, through a diaphragm of cellulose that is held in place by two perforated gold electrodes. In order to overcome the effects of polarization a quadrant electrometer was used.

It was pointed out, that for accurate measurements it is necessary to measure the specific conductivity of the liquid while it is in the diaphragm because "surface conductance" is quite an important factor in such electrokinetic studies.

III. DEVELOPMENT OF METHOD

Preliminary experimental work was carried on by a number of cataphoretic methods. However, these methods failed to give satisfactory results and were therefore discarded.

After a consideration of various other methods, it was decided to use, if possible, a cell similar to that used by Briggs and Gortner (1) in their studies on the electrokinetic potential at the cellulose-water interface. In their method, a liquid was forced through a diaphragm of cellulose that was held in place by two perforated electrodes. It was thought that such a cell could be constructed in which cream could be placed (being kept within the electrodes by a fine grade of filter paper) and skim milk could be forced

through, and any potential that might be established could be measured by suitable means. This method likewise proved very unsatisfactory. After a short time the filter paper would become clogged, thus preventing any streaming of the liquid.

Upon a suggestion of Gortner (10), it was thought possible to force the skim milk through a small hole in an electrode that was sealed in the end of a glass tube. A fine stream of skim milk could therefore be made to impinge upon another electrode that would serve as a target. The space in the immediate vicinity could then be filled with melted butter oil. Such a method, would in reality be the same as forcing skim milk through a fine "butter oil" capillary. Such a cell was constructed and a considerable amount of work carried on with it. However, it had the disadvantage of only being able to make measurements at comparative high temperatures, since it was essential that the butter oil be kept in the liquid state. Further, such an apparatus did not allow for complete adsorption of the milk colloids and salts at the interface and therefore the conditions were not identical with that of the normal fat globules as they exist in milk. Some difficulty also was experienced in keeping the pressure uniform.

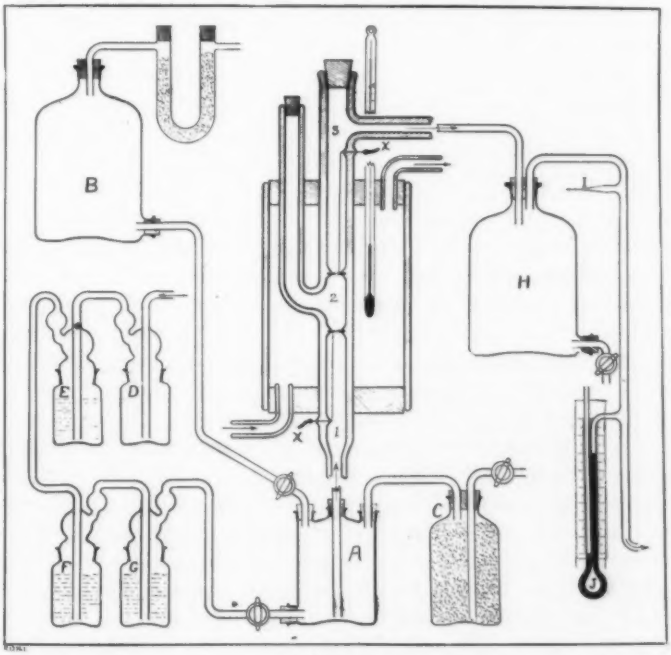

FIGURE 1

To faciiitate adsorption at the fat-liquid interface a special streaming potential cell was developed. A sketch of the apparatus is shown in figure 1. The capillary was formed by placing a fine, long needle through two platinum electrodes that were sealed in a heavy glass tube. The chamber (2) was then filled with melted butter oil which was then chilled to solidify it. By carefully removing the needle a butter oil capillary was thus formed. The capillary and chambers 1 and 3 were then filled with skim milk to allow for adsorption of the milk colloids to take place. After fifteen minutes the excess skim milk was removed by passing a stream of conductivity water through the cell. The preparation of the cell and the actual streaming potential measurements were made at a constant temperature by circulating water from a bath through the outer jacket.

In solutions which possess considerable conductivity the charge is partly neutralized; diluted solutions were therefore used. The sample which was to be subjected to streaming potential measurement was prepared by placing 10 cc. of a 1 to 1,000 dilution of skim milk in conductivity water, in Wolff bottle (A). To this, 100 cc. of conductivity water were added from reservoir (B). Air was now passed through wash bottles containing sulphuric acid (D), sodium hydroxide (E and F) and water (G), and admitted into bottle (A). This washed air was bubbled through the sample fifteen minutes to mix it, to remove any dissolved carbon dioxide, and to replace the air in the bottle. A controlled vacuum was then applied to draw the sample through the butter oil capillary. After passing through the capillary the liquid was collected in bottle (H). While liquid was being withdrawn from bottle A, air was admitted through a soda lime trap (C) rather than through the wash bottles so that the hydrostatic pressure could be accurately measured. The vacuum was regulated by the valve (I) and measured by manometer (J). Readings were taken at several different pressures to check the accuracy.

A sketch of the electrical apparatus used for measuring the potential and conductivity is shown in figure 2. The electromotive force (electrical leads marked X) that was set up at the ends of the capillary by the streaming liquid was measured by a type K-2 Leeds & Northrup potentiometer (P) and a carefully shielded Compton electrometer (E). In this procedure the potentiometer was first adjusted by balancing the standard cell (S.C.) against a two volt storage battery by means of the rheostat provided for that purpose. After this the galvanometer (G) was shorted out by means of switch (S4). Any desired voltage could then be taken from the potentiometer and opposed to the potential to be measured. With the electrometer (E) in the circuit of the unknown potential, the potentiometer voltage was varied until the electrometer indicated that the unknown potential had been exactly balanced. The potentiometer reading thus indicated the unknown potential. By using the electrometer as the null point instru-

ment polarization effects are eliminated or reduced to insignificance. The switches (S_1 and S_2) were provided for reversing the quadrants of the electrometer, and the poles of the cell respectively.

The conductivity measurements were made by means of the conventional Wheatstone-bridge method. To permit rapid switching from potential measurements to conductivity measurements the electrical leads X from the cell were connected to the switch (S_3). The high frequency current of one thousand cycles was produced by the microphone hummer (H); (Br) was a drum type Wheatstone bridge, (R) variable resistance and (T) the tunable telephones.

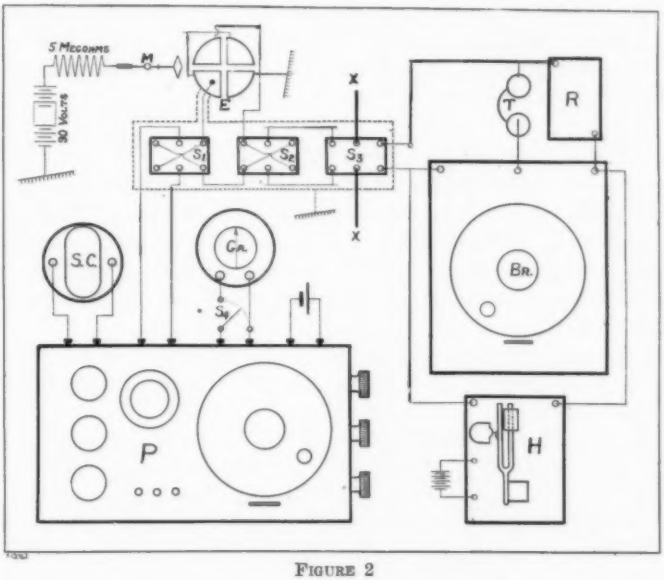

FIGURE 2

It will be noted that the results are not expressed in terms of the final zeta potential but as values of Hk_S/P (the observed electromotive force times the specific conductivity divided by the applied hydrostatic pressure). This expression was used since the remainder of the equation for calculating the zeta potential should be practically constant when such dilute solutions are used. However, it must be conceded, that in measurements made at lower temperatures, a slight error arises due to increased viscosity. Furthermore, since the values of the other terms of the equation for the solutions used were not accurately known, it was deemed advisable to express the results in terms of the above three mentioned variables.

Where hydrogen-ion concentrations were to be measured a Clark electrode vessel was used. To exclude carbon dioxide of the air, the vessel was

filled in the following manner: The funnel was closed with a two-holed rubber stopper which provided a vacuum connection and a connection to bottle (A). Hydrogen was passed through the vessel, through the funnel and the rubber tubing to replace all air. Suction was then applied to draw a suitable portion of sample into the funnel. By proper manipulation of the stop-cocks this sample was allowed to flow into the vessel and the excess hydrogen was allowed to escape at the opposite end. With the hydrogen in the vessel at atmospheric pressure, the vessel was shaken in the usual manner to establish equilibrium.

With highly dilute and poorly buffered solutions the usual potentiometric measurement leads to inaccurate results on account of the polarization involved in attaining the point of balance. This was avoided by using the same procedure for measuring this potential as has already been described for measuring the streaming potential.

IV. EXPERIMENTAL

The butter oil that was used in all of the experiments was prepared from unsalted, sweet cream butter taken directly from the churn. It was melted at approximately 45° C. to 50° C., care being taken never to heat it beyond the above mentioned temperatures. The curd was then allowed to settle to the bottom of the container and the oil was filtered twice through a standard grade of filter paper.

The skim milk was taken from the power separator in the University Creamery. All of the skim milk was diluted with one thousand parts of conductivity water.

Table 1 and figure 3 show the relationship between the applied hydrostatic pressure and the electromotive force that was set up. Theoretically these points should fall in a straight line, and as can be noted the points tend to form (within experimental error) a straight line.

In an effort to determine whether the charge was that of the fat plus the adsorbed proteins or just the fat, an experiment was run first measuring the potential at the fat-liquid interface. The capillary was then allowed

TABLE 1

Showing relation between pressure and electromotive force

PRESSURE	E.M.F.	H/P
mm.	*millivolts*	(−)
111	480	4.3
147	650	4.4
180	800	4.4
220	950	4.3
294	1280	4.3
333	1400	4.3
418	1800	4.3

to stand in contact with skim milk for a period of fifteen minutes, the unadsorbed material washed away, and the potential determined. The results are shown in table 2.

It was desired to determine the electrokinetic potential of samples of individual skim milk. The results are shown in table 3. As will be noted, the potential is negative and there is some variation even in mixed samples.

In order to make a comparison of the iso-electric points with those reported in the literature, that were determined by other methods, the

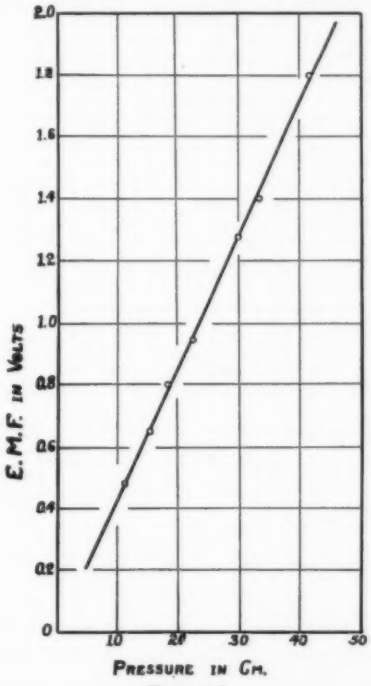

FIGURE 3

hydrogen-ion concentration at the point of zero potential was determined. The results are shown in table 4 and figure 4. It will be noted that the iso-electric point was found to be at approximately pH 4.3.

Tables 5 to 10 inclusive, and figures 5 and 6, show the effect of various salts on the magnitude of the electrical potential. It will be noted that the first four salts are all chlorides and that the valence of the other ion increases from one to four. The last two salts used contain the citrate and phosphate radical, respectively. The chloride salts tended to reduce the charge depending on the valence of the other ion. On the other hand, citrates and phosphates increased the already negative charge.

TABLE 2

Potentials at the fat-liquid interface compared with the fat-milk colloid-liquid interface

SERIES NO. 1

	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-6}$	$HK_s/P \times 10^{+6}$
	mm.	*millivolts*		*Mhos*	(–)
Fat-liquid	147	600	4.0	1.36	6.04
interface	180	750	4.1		
	220	900	4.0		
Fat-milk	147	380	2.5	1.90	4.75
colloid-liquid	180	450	2.5		
interface	220	600	2.5		

SERIES NO. 2

	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-6}$	$HK_s/P \times 10^{+6}$
	mm.	*millivolts*		*Mhos*	(–)
Fat-liquid	147	990	6.0	1.41	8.60
interface	180	1100	6.1		
	220	1400	6.3		
Fat-milk	147	600	4.0	2.01	7.84
colloid-liquid	180	700	3.8		
interface	220	850	3.8		

TABLE 3

Electrokinetic potentials of various skim milks from individual cows

SAMPLE	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-6}$	$HK_s/P \times 10^{+6}$
	mm.	*millivolts*		*Mhos*	(–)
	147	650	4.4	1.89	8.13
No. 1	180	800	4.4		
	220	900	4.0		
	147	365	2.4	2.11	5.48
No. 2	180	470	2.6		
	220	600	2.7		
	147	475	3.2	1.95	6.04
No. 3	180	600	3.3		
	220	660	3.0		
	147	600	4.0	1.90	7.41
No. 4	180	720	4.0		
	220	850	3.8		
	147	450	3.0	2.03	6.29
No. 5	180	570	3.1		
	220	690	3.1		
	147	510	3.4	1.96	6.84
No. 6	180	630	3.5		
	220	750	3.4		
	147	600	4.0	1.91	7.45
No. 7	180	700	3.8		
	220	875	3.9		
	147	390	2.6	2.10	5.67
No. 8	180	490	2.7		
	220	600	2.7		

Table 11 and figure 7 show the effect of temperature on the potential. It will be noted that almost a straight line function exists between the electrokinetic potential and the temperature of the measurement.

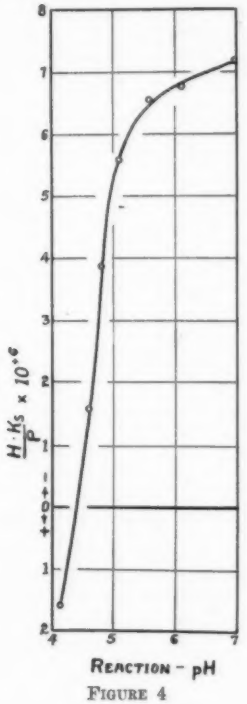

FIGURE 4

V. DISCUSSION

The experimental data reported here show that the potential at the fat-liquid interface is negative, and that it is slightly less negative after opportunity and time have been provided for the adsorption of milk colloids at the interface (Table 2). These results are in harmony with the findings of previous workers; the negative potential or charge on fat globules has been reported by several investigators (22, 27, 21); the effect of adsorbed milk colloids is indicated by the work of Prieger (27). Prieger determined the iso-electric point of fat globules in fat-water and fat-skim milk emulsions. She found that a lower pH was required to attain the iso-electric point in the fat-water emulsions than in the fat-skim milk emulsions.

It has been known for some time through cataphoresis experiments that a negative charge exists at the interface when oil drops are suspended in pure water. Since pure water contains only hydrogen and hydroxyl ions,

TABLE 4

pH	PRESSURE	E.M.F.	H/P	$K_a \times 10^{-6}$	$HK_a/P \times 10^{+6}$
	mm.	*millivolts*		*Mhos*	
	147	675	4.5	1.56	7.02 (−)
	180	800	4.4		
	220	1000	4.5		
	147	590	4.0	1.71	6.84 (−)
6.14	180	725	4.0		
	220	875	3.9		
	147	490	3.3	2.01	6.63 (−)
5.63	180	600	3.3		
	220	725	3.2		
	147	300	2.0	2.83	5.66 (−)
5.10	180	350	1.9		
	220	350	2.0		
	147	200	1.3	3.05	3.96 (−)
4.81	180	235	1.3		
	220	310	1.4		
	147	75	0.5	3.31	1.65 (−)
4.68	180	100	0.5		
	220	120	0.5		
	147	60	0.4	4.02	1.60 (+)
4.12	180	75	0.4		
	220	90	0.4		

TABLE 5

Effect of potassium chloride

CONC.	PRESSURE	E.M.F.	H/P	$K_a \times 10^{-6}$	$HK_a/P \times 10^{+6}$
$N \times 10^4$	*mm.*	*millivolts*		*Mhos*	(−)
	147	445	3.0	1.20	3.60
0.0	180	520	2.8		
	220	650	2.9		
	147	470	3.2	1.25	4.00
0.5	180	560	3.1		
	220	700	3.1		
	147	500	3.4	1.31	4.32
1.0	180	600	3.3		
	220	725	3.3		
	147	450	3.0	1.37	5.11
2.0	180	550	3.0		
	220	685	3.1		
	147	400	2.7	1.96	5.29
4.0	180	490	2.7		
	220	575	2.6		
	147	300	2.0	2.1	4.2
6.0	180	360	2.0		
	220	450	2.0		

TABLE 6
Effect of calcium chloride

CONC.	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-6}$	$HK_s/P \times 10^{+6}$
$N \times 10^4$	*mm.*	*millivolts*		*Mhos*	(–)
	147	450	3.2	1.11	3.50
0.0	180	575	3.2		
	220	700	3.1		
	147	385	2.6	1.23	3.07
0.5	180	450	2.5		
	220	550	2.5		
	147	290	1.9	1.29	2.45
1.0	180	330	1.8		
	220	430	1.9		
	147	75	0.5	2.32	1.39
2.0	180	110	0.6		
	220	140	0.6		
	147	60	0.4	2.98	1.19
4.0	180	80	0.4		
	220	90	0.4		
	147	30	0.2	3.37	1.01
6.0	180	70	0.3		
	220	80	0.3		

TABLE 7
Effect of ferric chloride

CONC.	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-6}$	$HK_s/P \times 10^{+6}$
$N \times 10^4$	*mm.*	*millivolts*		*Mhos*	(–)
	147	500	3.4	0.96	3.36
0.0	180	630	3.5		
	220	775	3.5		
	147	400	2.7	1.05	2.83
0.5	180	480	2.6		
	220	600	2.7		
	147	300	2.0	1.18	2.36
1.0	180	370	2.0		
	220	450	2.0		
	147	250	1.7	1.22	2.07
2.0	180	300	1.6		
	220	375	1.7		
	147	220	1.5	1.28	1.92
4.0	180	275	1.5		
	220	350	1.6		
	147	170	1.1	1.31	1.44
6.0	180	200	1.1		
	220	250	1.1		

TABLE 8
Effect of thorium chloride

CONC.	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-6}$	$HK_s/P \times 10^{+6}$
$N \times 10^4$	*mm.*	*millivolts*		*Mhos*	
	147	600	4.0	0.98	4.02 (−)
0.0	180	750	4.1		
	220	950	4.3		
	147	500	3.4	1.07	3.85 (−)
0.5	180	700	3.8		
	220	800	3.6		
	147	330	2.2	1.21	2.66 (−)
2.0	180	400	2.2		
	220	500	2.2		
	147	200	1.3	1.28	1.66 (−)
4.0	180	265	1.4		
	220	300	1.3		
	147	.....	.....	1.32	0.13 (+)
6.0	180	10	0.05		
	220	22	0.10		

TABLE 9
Effect of di-sodium phosphate

CONC.	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-6}$	$HK_s/P \times 10^{+6}$
$N \times 10^4$	*mm.*	*millivolts*		*Mhos*	(−)
	147	525	3.5	1.01	3.54
0.0	180	625	3.4		
	220	775	3.5		
	147	650	4.4	1.14	4.90
0.5	180	750	4.1		
	220	950	4.3		
	147	740	5.1	1.22	6.10
1.0	180	900	5.0		
	220	1100	5.0		
	147	620	6.2	1.56	9.51
2.0	180	1100	6.1		
	220	1350	6.1		
	147	1030	7.0	2.03	14.21
4.0	180	1280	7.1		
	220	1550	7.0		
	147	810	5.5	2.27	12.48
6.0	180	1000	5.5		
	220	1200	5.4		

the negative potential is attributed to more extensive adsorption of hydroxyl ions than of hydrogen ions. In a fat-skim milk emulsion we have, in addition, the various ions of the milk salts, and the milk colloids. The potential that obtains at the interface must be the net result of the adsorption of the various ions, the adsorption of each ion reaching equilibrium at a definite concentration of that ion in the serum proper (reversible adsorption).

TABLE 10
Effect of sodium citrate

CONC.	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-5}$	$HK_s/P \times 10^{+5}$
$N \times 10^4$	*mm.*	*millivolts*		*Mhos*	(–)
	147	650	4.4	1.07	4.70
0.0	180	800	4.4		
	220	990	4.5		
	147	750	5.1	1.19	5.72
0.5	180	970	5.3		
	220	1160	5.2		
	147	900	6.1	1.80	11.34
1.0	180	1175	6.5		
	220	1400	6.3		
	147	1000	6.8	2.21	15.25
2.0	180	1300	7.2		
	220	1500	6.8		
	147	1120	7.6	2.33	17.71
3.0	180	1400	7.7		
	220	1650	7.5		
	147	900	5.9	2.87	17.22
5.0	180	1110	6.1		
	111	675	6.0		
	147	750	5.1	3.01	15.05
7.0	180	900	5.0		
	220	1025	4.9		
	147	300	2.0	4.63	9.26
10.0	180	360	2.0		
	220	.....	.....		

However, it must be expected that the conditions will be further complicated by the fact that some of the milk colloids are irreversibly adsorbed, thereby conferring new properties upon the interface. With a film of irreversibly adsorbed milk colloids on the fat globules ("fat hulls") we now have an interface in which the adsorbed milk colloids will probably play the dominant rôle in determining the potential.

That fat globules in milk possess "fat hulls" in the sense that there is irreversibly adsorbed material present has been demonstrated by the work of various investigators. In such work the method of isolating the "fat hull" material has in all cases involved the repeated washing of the globules with water, followed by the removal of the fat, leaving the "fat hull" material for study. In washing the fat globules, two methods have been used; in one method the fat globules, introduced as whole milk into the bottom of a tall column of water, are washed as they rise through the water; in the second method milk is separated centrifugally, the cream is suspended in

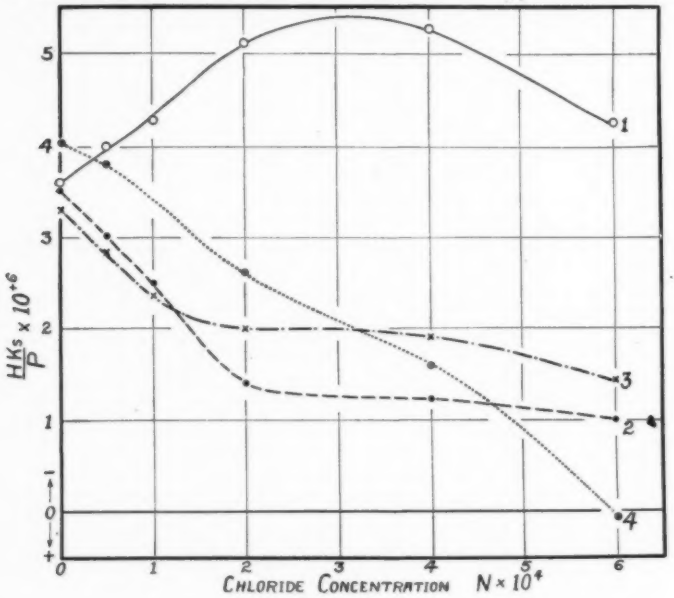

FIGURE 5
1 = KCl
2 = $CaCl_2$
3 = $FeCl_3$
4 = $ThCl_4$

water and reseparated a number of times. In either method the fat globules are suspended in pure water for a sufficient length of time that reversibly adsorbed material must inevitably be removed. The study of "fat hulls" in the past has therefore included only material that is irreversibly adsorbed.

The chemical nature of "fat hull" material in milk has been the subject of numerous investigations since 1840, and divergent conclusions have been reached. The material has been variously reported as casein (31), glyco-

protein (30), a mixture of globulin-like proteins and phospholipids and so forth. Palmer and Wiese (32) in a recent publication point out that the material consists of a mixture of proteins and phospholipids, and that the protein possesses the properties of both a hydrophyllic and a hydrophobic colloid. They point out further that its physical properties, percentages of N, S, and P, do not correspond with any of the other milk proteins. It

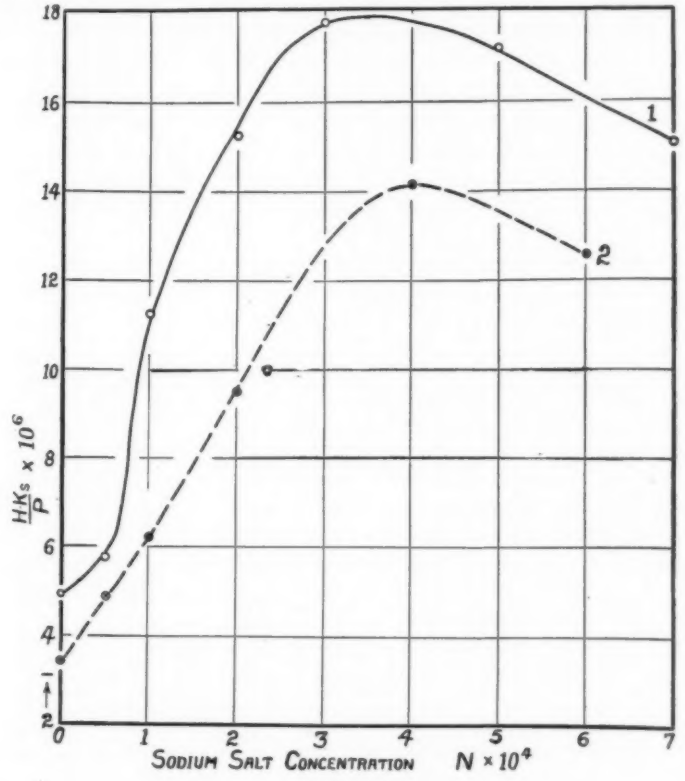

FIGURE 6
1 = Sodium Citrate
2 = Na_2HPO_4

should be pointed out, however, that their comparisons of S, N, and P contents were with analytical data taken from the literature rather than with analyses of milk proteins that had been subjected to the same special treatment that they employed in preparing the "membrane" protein. Nor do they consider the data of Titus, Sommer and Hart (31) which shows good agreement between the "membrane" protein and casein with respect to

nitrogen distribution, content of sulphur, phosphorus and tryptophane, specific rotation, and the precipitin reaction. The latter investigators concluded that the "hull" material consisted chiefly of casein.

For the present purpose it is sufficient to consider that there is a protein adsorbed at the fat-serum interface, and that this must necessarily alter the conditions with respect to the electrokinetic potential. Similarly, adsorbed phospholipids, postulated by Palmer and Samuelson (24), must exert an effect. That the adsorbed material alters the potential is shown by table 2, which shows that the electrokinetic potential is lower (less negative) after

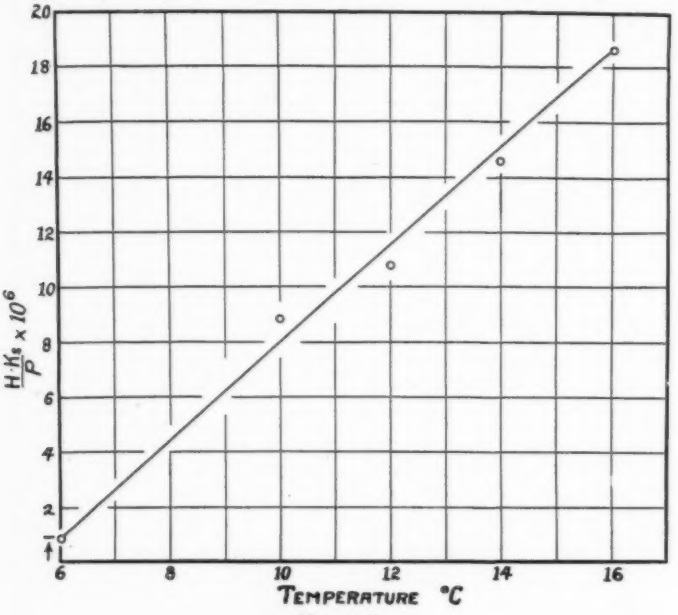

FIGURE 7

opportunity and time have been provided for adsorption. The work of Prieger (27) shows an isoelectric point of pH 2.27 – 3.07 for fat globules suspended in water, and pH 4.21 in milk. Since such reactions are on the acid side of the iso-electric point as commonly found for milk proteins (pH 4.6 – 4.7), the proteins by themselves should have a positive potential. Such proteins adsorbed on the fat globules should bring the iso-electric point of the globule nearer to pH 4.6. In the present work the iso-electric point or the point where the potential at the fat-serum interface is zero was found to be pH 4.3 (Table 4 and Figure 4).

The potential at the interface must be the net result of adsorbed ions and special properties conferred by irreversibly adsorbed material. The

TABLE 11
Effect of temperature

° C.	PRESSURE	E.M.F.	H/P	$K_s \times 10^{-6}$	$HK_s/P \times 10^{+6}$
	mm.	*millivolts*		*Mhos*	(–)
	147	175	1.1	0.90	0.99
6.0	180	200	1.1		
	220	250	1.1		
	147	470	2.6	1.60	2.86
8.0	180	600	2.7		
	220	740	2.6		
	147	750	5.0	1.82	8.92
10.0	180	900	5.0		
	220	1050	4.7		
	147	810	5.5	2.2	10.8
12.0	180	975	5.4		
	220	1200	5.4		
	147	.....	.....	2.18	14.60
14.0	180	1200	6.6		
	220	1500	6.8		
	147	1225	8.3	2.24	18.59
16.0	180	1500	8.3		
	220	.....	.....		

special properties contributed by the irreversibly adsorbed materials involve adsorption of ions by it and the dissociation of the irreversibly adsorbed material itself. Since proteins are ampholytes, the following conditions apply with respect to reaction:—

Protein dissociated as a base, therefore the potential will be progressively more positive as the acidity is increased ← Increasing Acidity — Iso-electric Protein — Decreasing Acidity → Protein dissociated as an acid, therefore the potential will be progressively more negative as the acidity is decreased or the alkalinity is increased

(B) (A)

This reaction must be expected to affect the potential because (1) it fixes the hydrogen- and hydroxyl-ion concentrations and their direct absorption (2) it determines the type and extent of dissociation of the irreversibly adsorbed protein, and (3) it determines the degree of dissociation and valence of polyvalent ions such as citrate and phosphate ions.

The ions furnished by salts must influence the potential by (1) the direct adsorption of the ions, and (2) the effect of the ions on the dissociation of the adsorbed protein. Under (A) above the proteins yield compounds such as sodium proteinate, potassium proteinate, etc. The extent

to which these compounds are dissociated must, in conformity with the mass law, be affected by the concentration of the cations. Thus:

$$A_n \text{ Proteinate} \rightleftarrows A_{(n-x)} \text{ Proteinate}^{x-} + xA^+$$

A representing any cation.

The application of the mass law to this problem cannot be made in a quantitative manner, but in a qualitative sense it must be applicable. Any complete explanation, no doubt, must involve a number of additional factors, such as the effect of double bonds and natural salt or ketopiperazine structures within the protein molecule.

The effect of added salts was in general in harmony with expectations. The chlorides (with the exception of KCl which first increased and then decreased the potential) all decreased the electrokinetic potential. Since in these salts the chloride ion is common to all, the cation should, therefore, be the effective ion, the efficiency increasing with the valence. In the case of the Thorium ion which is tetra-valent, the charge was actually reversed.

The phosphate ion was slightly less effective than the citrate ion in making the charge more strongly negative. This is to be expected from the dissociation constants of the phosphoric acid and the citric acid, which are as follows: phosphoric acid, $k_1 = 1.1 \times 10^{-2}$, $k_2 = 1.95 \times 10^{-7}$, $k_3 = 3.6 \times 10^{-13}$; citric acid, $k_1 = 8.3 \times 10^{-4}$, $k_2 = 4.1 \times 10^{-5}$, $k_3 = 3.2 \times 10^{-6}$. The titration curves of phosphoric and citric acid, fixed by these constants, show that at reactions involved in these and similar studies, the citric acid is more completely neutralized than the phosphoric acid. For example at pH of 6.5, soluble phosphates exist in the proportion of 3.12 moles of primary phosphate to 1.95 moles of secondary phosphate. In considering the effect of added phosphates at this reaction (even if the addition had been as tertiary phosphate, (Na_3PO_4) the phosphate ion should, therefore, be considered partly mono-valent and partly di-valent. A similar calculation for citrates can not be given with the same degree of accuracy because the three dissociation constants for citric acid are more nearly of the same value; but inspection of the dissociation constants and the titration curve for citric acid, clearly shows that the citrates will exist partly as the di-basic and partly as the tri-basic salt at pH 6.5. These facts are pointed out in some detail, because the error is frequently made of considering citrates and phosphates as furnishing tri-valent ions regardless of the reactions.

Temperature is known to affect the dissociation of acids, bases, salts and of water itself, and the activity of the ions. It is therefore to be expected that temperature should affect the electrokinetic potential. As is shown in table 11, increasing the temperature increases the negative potential quite rapidly. In a complex system such as milk it is difficult to attribute this effect to a single factor or several specific factors; however, it is likely that the following considerations are involved: (1) Since hydroxyl ions are more strongly adsorbed than hydrogen ions, the increased

dissociation of water with higher temperatures should favor the greater adsorption of hydroxyl ions. (2) Higher temperatures are likely to increase the dissociation of the adsorbed protein as an acid. (3) The increased dissociation of the salts with increased temperatures is likely to be most significant with the citrates and phosphates, especially the latter; increased dissociation in this case would increase the valence of the ions (*e.g.*, phosphate ions changed in the direction of primary to secondary to tertiary). (4) Increased temperatures decrease the hydration of the irreversibly adsorbed proteins thereby moving the boundary which is involved in the electrokinetic potential closer to the globule itself.

The factors which are shown to affect the electrokinetic potential at the fat-serum interface must also be expected to affect the potential or charge on the colloidal protein particles themselves. This must follow if the adsorbed proteins play the dominant rôle in determining the charge on the fat globules. As far as can be stated at the present time, the effects of salts on the stability of milk proteins, as reported by Sommer and Hart (29), are in harmony with this expectation. It is further supported by the work of Doan (4) (5) (6) which shows that there is a relationship between the clumping of fat globules and the stability of proteins.

SUMMARY

1. A method is described, based on the theory of streaming potentials, that may be used in measuring the magnitude of the electric charge at the fat-serum interface.

2. The stability of colloidal systems is discussed and a mathematical development of the streaming potential formula is given.

3. Data are presented to show that an interface quite comparable to that of the fat globule as it exists in milk is being considered.

4. The electrokinetic potentials observed using various samples of milk from individual cows are shown to vary over a comparatively wide range.

5. The iso-electric point of the interface was found to be at about pH 4.3.

6. Potassium chloride, added in increasing amounts, caused an increase that was later followed by a decrease in potential. Chlorides of calcium, iron and thorium decreased the electrokinetic potential. Thorium was found to be the most effective and at certain concentrations reversed the sign of the charge.

7. Di-sodium phosphate and sodium citrate caused the potential to become more strongly negative. The latter salt was found to be the most effective.

8. Increasing temperatures produced a very pronounced increase in the observed electrokinetic potential.

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X-RAY INVESTIGATION OF THE MICROCRYSTALLINE STRUCTURE OF BUTTER-FAT

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It may be expected that one of the factors which have an influence on the firmness of butter will be the way in which and to what extent butter-fat crystallizes. A far advanced crystallization can be obtained by holding the cooled cream before churning, or the butter for a long time. By following the first method the fresh butter will be much more solid than butter made without cooling the cream beforehand. As a result hereof it can be worked more and drier and will give a finer distribution of the liquid; moreover, less fat will get lost in the buttermilk. Although it may be assumed that in this butter the fat is practically in equilibrium at the churning temperature, the butter still strongly solidfies when kept at the same temperature, which behavior, therefore, cannot be ascribed to a further crystallization. It is, however, not impossible that a variation in the size of the crystals is the cause of this phenomenon, or that some other change in microstructure occurs. Also for other plastic substances this question seems not yet to be solved.

The cooling of butter prepared from cream that has not been held cooled beforehand differs from the cooling of butter from chilled cream. At first, the fat of this butter is not in equilibrium as the greater part is still in the non-crystallized state: on cooling, this part will slowly solidify in the butter with a chance of forming crystallites of another size. Thus, in this case, the way in which the crystallization takes place and which we discussed above, would be different from that which occurs in the first mentioned butter. When, by following the two methods indicated, summer-butter is prepared from the same cream and the butters are kept at a temperature of about 9° C., then the original great difference in firmness almost completely disappeared after a week; it even happens that the product, which was softer when fresh, turns out to be the more solid one later on. Yet in most cases the body of the butter, prepared from cream that was cooled beforehand, is better, as it proves to be more "elastic"; the other sort, on the contrary, is not elastic, especially not when the churning temperature has been rather high.

The size of the fat-crystallites can be influenced also in a different way, namely by varying considerably the temperature of the washing-water. When this temperature is chosen considerably lower than the churning tem-

[1] The X-ray part of the investigation.

perature, *i.e.*, lower than about 6° C.,[2] then a rapid crystallization will take place, since the fine butter grains will also easily assume this temperature. When, however, water is used, which is but 1 or 2° colder than the churned product and the butter is cooled afterwards, then the cooling of the butter will take place slowly again, thus causing another way of crystallization. When following this method, the difference in structure can be seen very distinctly on the fracture with the unaided eye. The butter rinsed with very cold water shows a fine texture, the other a coarse, more normal structure.

From the aforesaid it becomes evident that a more exact knowledge of the microcrystalline structure of butter-fat would be very important in practice and this is the reason why we have endeavored to investigate to what extent an X-ray investigation would be of use. Although our experiments in this direction have not yielded the results we expected from them, we thought it useful to present them.

Some general remarks as to the method of procedure which was followed for the X-ray investigation may be given first.

X-ray interference exposures were made of all sorts of butter-fat to be investigated according to the method of Debye-Scherrer. To this end the material (see fig. 1)[3] is fastened in the form of a thin rod P to the axis of

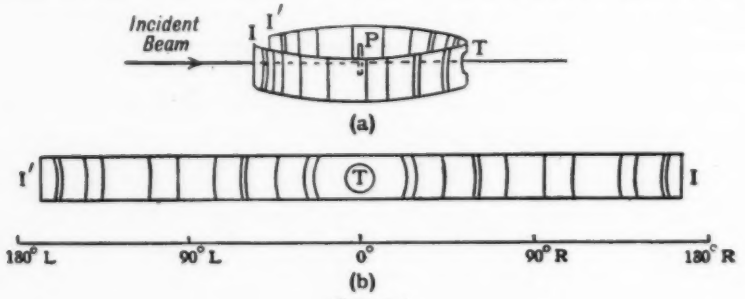


FIGURE 1

a cylindrical camera and exposed to a narrow beam of monochromatic X-rays, which enters the camera through a tubulated diaphragm in the wall. In case of microcrystalline material, containing a large number of crystallites with arbitrary position of their axes with regard to the incident X-ray beam (as will be generally the case in any crystal powder), the scattered rays, due to interference of the incident beam with the crystal lattice planes, form a system of circular cones round this beam as an axis. These cones intersect a film I–I′, which is placed along the wall of the

[2] Hildesh. Molkerei. Zeit. 43: 1198, 1929.

[3] From: W. L. Bragg, The Crystalline State, London, Bell & Sons, 1933, where a modern exposition is given of the theory and the method of X-ray investigation.

cylindrical camera, according to curved lines which, in first approximation, form a system of concentric circles (in general, as a consequence of the limited height of the film, circular *arcs*) with the point T, where the incident beam of X-rays strikes the film, as the common center. From the distance of the circles and the diameter of the camera, the top angle, 4θ, can be calculated for each cone and from this, knowing the wave length λ of the X-ray light used, by means of Bragg's well-known formula $\lambda = 2d \sin \theta$, the distance of periodicity d of the crystal lattice planes, corresponding with each special circle.

From the character of the interference lines, data concerning the size of the crystallites can be derived in certain cases: when this size is larger than about 10μ, the lines are no more continually blackened, but the rays scattered by the individual crystallites give rise to separate points on the circles; when, however, the crystallites are smaller than 0.1μ, the lines will be considerably broadened as a result of unsufficient resolving power.

Finally it may be remarked that a liquid phase gives rise to one or more very diffuse interference circles (halos) with a diameter related to the mean distance of the molecules in the liquid, which latter, as a result of thermal agitation, varies constantly.

The photographs reproduced hereafter were obtained with the Philips Metalix apparatus for the investigation of crystal structure.[4] To obtain a preparation of the butter, suitable for an exposure, a capillary tube (total diameter $\frac{1}{2}$–1 mm) with thin walls, for X-rays transparent glass, is dipped several times in the butter, till it contains a sufficient quantity. In order the prevent the temperature of the butter fat to be influenced by this manipulation, especially in view of the very small quantity required for an exposure, the capillary was kept close to the butter beforehand, so that both had the same temperature; then it was taken up with cottonwool or gloves to reduce warming by the hand. The preparations were then put in the center of a Debye-Scherrer camera (diameter 57.3 mm) belonging to the above-mentioned apparatus for crystal structure and exposed during half an hour with copper-Kα-radiation ($\lambda = 1.54$ Å). A thermometer was entered through an opening in the cover of this camera, the reservoir close to the preparation, so that the temperature of the latter could be controlled during the exposure. In special cases, where it was not allowed to surpass a certain maximum temperature, cooled air was blown through side tubes, mounted in two diametrically opposite points of the camera wall, with such velocity that the thermometer indicated the desired temperature.

1st Experiment: Crystallized butter-fat in comparison with butter.

After some preliminary experiments, exposures were made of butter-fat of very high and of very low melting point in comparison with ordinary

[4] A. Bouwers and W. Busse, ZS. f. Krist. 77, 507, 1931.

butter as such. In order to obtain the two first sorts, a liquid part was separated at 11° C. from filtered butter-fat, the solid rest being melted and then crystallized at about 32° C.; then the crystals were filtered and washed with a mixture of alcohol-petroleum ether, the rests of which are removed from the crystals by introducing dry carbonic acid in the molten mass at a mercury pressure of about 5 mm.

The resulting X-ray photographs are reproduced in fig. 2 *A*, *B* and *C*.

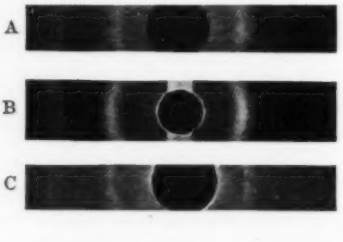


FIGURE 2

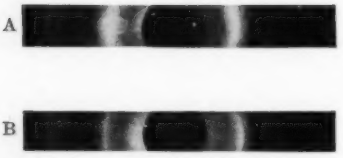


FIGURE 3

A relates to the fat with low melting point ("liquid oil"), *C*, to that with a high melting point ("solid fat"), whereas *B* represents an exposure of ordinary butter at about 18° C. The photographs are in agreement with what might be expected from the above given general remarks: *A* shows one diffuse circle (halo), which is characteristic for a liquid phase, *C* some (2 are clearly visible) relatively sharp interference lines, as can be expected for a solid crystalline material.

In connection with the known diameter of the camera and the wave length of the X-ray radiation used, it is found from the distance of the lines that these correspond with periodicity distances in the crystal lattice of resp. 4.32 and 3.87 Å, that are distances of the order of magnitude as are generally found for the "cross-dimensions" of molecules with long chains of carbon atoms.[5] Photograph *B* of the butter preparation can be considered as a superposition of the photographs *A* and *C*; it shows the "oil-ring" as well as the "solid crystal lines" (only the outmost of these two is

[5] See *e.g.* ZS. f. Krist.: Strukturbericht 1913–1928; the much larger periodicity distance, which corresponds with the *length* dimensions of the fat molecules, gives rise to an interference cone with very small top angle. In connection with the dimensions of the camera, this cone cuts the film along so small a circle that the latter falls within the opening in the middle of the film, through which the direct beam leaves the camera. Conquently this interference circle cannot appear on the film.

visible separately, as the inmost line just coincides with the exterior part of the oilring; compare also fig. 3). *Thus the photograph shows very distinctly the presence of liquid fat as well as of solid fat in the butter.*

FIGURE 4

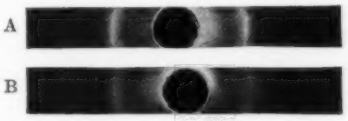
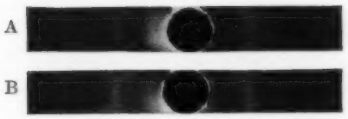


FIGURE 5



2nd Experiment: Influence of keeping butter at low temperature on its crystallization.

The occurrence of the "oilring" in experiment I led to the next experiment.

A portion of summer-butter, prepared from cream, which had not been cooled to a very low temperature, was exposed immediately after preparation (fig. 3 *A*), whereas another portion was kept during two months at a temperature of $-10°$ to $-12°$, after which a photograph was made (fig. 3 *A*).

A comparison of these two photographs shows very distinctly that, as a result of keeping the butter at low temperature during a long time, a strong crystallization of solid butter fat has taken place; the "oilring," still distinctly visible on photo 3 *A*,[6] has almost completely disappeared on photo 3 *B*, and has been replaced by the two "crystal-lines." From the fact that these lines, although close together on the film,[7] differ apparently considerably in width, it seems probable that line-broadening as a consequence of the smallness of the crystallites occurs and that these crytallites, at least as regards one of their cross-dimensions, are smaller than 0.1μ.

3rd Experiment: Influence of wash-water of a very low temperature in comparison with that of normal temperature on the crystallization of butter.

Two equal portions of sour cream (cooled after pasteurization at about 9° C., soured at 13° C.) were churned in March and one portion of the butter washed with very cold water, the other in the normal way:

[6] Unfortunately the left side of this photograph has been damaged.

[7] As, namely, the line broadening is dependent on the reflection angle θ, only lines which lie close together on the film (thus differing little in θ) may be compared with each other.

	Cold rinsing	Warm rinsing
churning temperature	13.3°–14.5°	13.3°–14.5°
duration of churning	32′	35′
temperature washing-water	*3.6°*	*13°*
temp. butter after first working	6.8°	13.2°
temp. butter after second working	9°	12.8°
number of revolutions	36	18

That same day Debye-Scherrer exposures were made of both sorts of butter (fig. 4 *A* and *B*) and this was repeated after they had been kept during a week at a temperature of 8° (fig. 5 *A* and *B*). After this week the consistency, which, of course, considerably differed in fresh condition, proved to be almost equal after heating up to 17° C.; with the Perkins-apparatus the following results were obtained for hardness:

"cold rinsing," at 16.8° C.	0.23
"warm rinsing," at 16.7° C.	0.24

The difference in structure mentioned above was very well visible on the fracture and the coldly washed product showed the well-known appearance of solidified limiment.

Inspection of the photographs does not show a marked difference between the two sorts of butter, neither in fresh condition nor after a week's storage at 8°. Although the method of exposure is not very "sensitive" with regard to the detection of small differences in size of the crystallites, it can yet be concluded from this fact, that the size of the crystals in the two kinds of butter does not differ considerably and consequently it does not seem very likely that the marked difference in structure must be ascribed exclusively to a difference in crystal size. However, it must be kept in mind that, as indicated already above, the interference circle due to the periodicity distance of the crystal lattice corresponding with the *length*-dimension of the fat-molecules, is *not* present on the photographs: the possibility thus remains that the dimensions of the crystallites parallel to the length-direction of the molecule show larger differences (so that they would give rise to interference circles which differ distinctly in sharpness).

Finally it may be remarked that an eventual marked anisodiametrical form of the fat-crystallites (rods or plates) may give rise to "performance orientations" during the working. The fact that preference orientations can occur when spreading fat is proved by experiments of J. J. Trillat.[8] A difference in "habitus" of the crystallites is in all probability accompanied by a difference in "orientation power" and might eventually come to expression as a difference in external structure of in elasticity.

[8] J. J. Trillat, C. R. de l'Acad. d. Sc., **182**, 843, 1926; Ann. d. Physique [10] **6**, 5, 1927; see also: "Les applications des Rayons-X" (Les Presses Universitaires de France, Paris, 193).

A CHART TO AID IN SCORING MILK FLAVOR

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In the summer of 1933, a project was inaugurated at the Oklahoma station to study certain phases of the question of flavors in milk. At the outset it was recognized that work of this nature depended to a considerable extent on the accuracy of the scores accorded the milk samples. It was further realized that the scoring of milk can not give wholly exact and uniform results but is subject to considerable variation among individual judges. Even a given judge, though entirely proficient, is by no means infallible in such a delicate matter as the evaluation of milk flavor.

In contemplating the procedure to be employed in obtaining the milk scores in this project two alternatives presented themselves. On one hand, there was the possibility of using as judges two of the staff members who had acquired considerable experience in scoring and judging work. On the other hand, there were four additional staff members available who could assist the two experienced men.

It was finally decided the use of all six men would be the more suitable procedure in such a study. There appeared to be considerable advantage from the standpoint of accuracy, in securing the greater number of individual scores. Also the greater number of persons whose scores were secured could more nearly represent the view-point of consumers. The significance of this view-point is not to be minimized in studies involving milk quality.

On starting the work there immediately arose the need for some device to aid, especially the inexperienced men, in scoring the milk for flavor. These men sought first of all a proper vocabulary so they could reveal the flavors they detected. They as well as the experienced judges wanted some guidance as to the seriousness of each flavor defect. To accomplish these objectives the chart as shown here was prepared and displayed in the laboratory where the scoring was done.

Efforts in formulating guides in scoring milk for flavor have been made by other workers. Babcock and Leete[1] proposed a "general guide for scoring flavor and odor." Lucas[2] also has suggested certain numerical scores for various flavor defects in milk.

The rules for the students' National contest in judging dairy products carry a detailed milk score card in which 15 flavor defects are listed. This

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[1] Babcock, C. J., and Leete, C. S. How to conduct milk and cream contests. U. S. Dept. Agr. Cir. 384: 18. 1929.

[2] Lucas, P. S. Many factors cause abnormal milk flavors. Mich. Exp. Sta. Quar. Bul. XII: 1. 1929.

material while providing terms that may be used to describe a defect affords no guidance as to the score that should be accorded when the defect varies in intensity.

THE PROPOSED CHART

In the chart here are included the five classes into which milk is divided on a basis of flavor as suggested by Babcock and Leete. The perfect score for flavor is 25. Milk that scores 23 or above, is designated as *excellent*, 21 or 22 *good*, 18, 19 or 20 *fair*, 12 to 17 inclusive *poor*, and 11 or below is *bad*. A fractional score in no case serves to raise a sample into a higher class than it would enter without the fraction. For instance, a sample scoring 20½ is classed as fair, not as good. Eighteen different defects in flavor are enumer-

Flavor Defects and Suggested Scores
(s. indicates slight; v. indicates very)

CLASS	EXCELLENT	GOOD	FAIR	POOR	BAD
SCORES	23 and above	21 and 22	18, 19 and 20	12 to 17, incl.	11 and below
Acid	.....	.....	.....	s. acid	acid v. acid
Bitter	.....	.....	.....	s. bitter and bitter	v. bitter
Cooked	.....	s. cooked	cooked	v. cooked	.....
Cowy	.....	s. cowy	cowy	v. cowy	.....
Disinfectant	.....	.....	.....	s. disinfectant	disinfectant and v. disinfectant
Feed	.....	s. feed	feed	v. feed	.....
Flat	.....	s. flat	flat	v. flat	.....
Metallic	.....	.....	s. metallic	metallic and v. metallic	.....
Musty	.....	.....	.....	s. musty	musty and v. musty
Nutty	.....	.....	s. nutty	nutty and v. nutty	.....
Oxidized	.....	.....	s. oxidized	oxidized and v. oxidized	.....
Rancid	.....	.....	.....	s. rancid	rancid and v. rancid
Salty	.....	.....	s. salty	salty	v. salty
Sharp	.....	s. sharp	sharp	v. sharp	.....
Stale	.....	.....	s. stale	stale and v. stale	.....
Sweet	.....	s. sweet and sweet	v. sweet	.....	.....
Watered	.....	.....	s. watered	watered and v. watered	.....
Weedy	.....	.....	.....	s. weedy	weedy and v. weedy

ated. It is recognized that some of these terms are subject to challenge. Again some judges would choose to add other terms. In a year's work with this chart, during which time about 1,000 milk samples have been scored by five or six judges, a few suspected off-flavors other than the eighteen enumerated have been encountered. However, none of these other flavors

has been observed with enough certainty or frequency to justify its inclusion in the chart.

In formulating this chart it was believed each flavor could be resolved into at least three degrees of intensity. Accordingly the terms "slight" and "very" are employed to reveal the upper and lower gradations in the flavor. These terms, or comparable ones, are used by all judges. The effort in the chart is to ascribe some numerical significance to the different gradations.

It is observed in several cases here that two gradations of a given flavor are suggested under the same class. For instance, milk that is either "acid" or "very acid" is classed as *bad*. Again, milk that is "slight bitter" is held down into the same class as "bitter" milk. In devising the chart decisions that relegated the different flavor gradations into the various classes were based on experience and usual practice in scoring milk. They are entirely arbitrary and no effort is made here to defend them. On the whole, however, it is believed few workers would have occasion to deviate greatly from the suggestions as given.

Frequently in using this chart the judges have found occasions which prompted them to use an additional gradation to reveal a "very slight" intensity in some flavor. Such occasions have arisen most frequently with the salty samples. It is suggested in the chart that milk found to be "slight" salt should be classed as *fair*. However, the judges sometimes detected a very slight taste of salt that was in no wise objectionable. They felt it was unduly critical to hold the milk down to *fair* so used the gradation "very slight" salt and kept the milk in the *good* class.

OBSERVED BENEFITS IN USING THE CHART

In the year's use of the chart during which time about 1,000 samples have been scored by five or six judges some observations thought to be of value have been made.

1. The use of the chart contributes somewhat to the unification of the scores accorded by individual judges. Of course, it does not make for anything like perfect unanimity. Variation in the scores will exist unless the judges confer and coordinate their criticisms of each sample.

2. In work of this nature involving studies of milk quality, the procedure wherein several judges, under such guidance as the chart provides, express their individual opinions is believed preferable to the plan wherein the judges confer to attain greater uniformity in the scores. The former plan can be related to the consumers' view-point more directly than the latter.

3. The use of the chart provides an incentive for the judges to score inferior milk sufficiently low so it is effectively differentiated from superior milk. It is believed that usual practice in scoring milk is characterized by

an unduly critical attitude toward superior milk and undue liberality toward inferior milk.

4. Such a chart fortifies one's vocabulary so he feels greater security and confidence in scoring milk.

5. It affords, not only amateurs but experienced judges as well, some guidance as to the seriousness of various flavor defects in milk. The recognition of the five classes of milk based on flavor, namely; excellent, good, fair, poor and bad seems particularly helpful.

6. The chart is conducive to a "flavor consciousness" among men who are interested in problems relating to milk, such as college and short course students and plant men.

7. It proves somewhat fascinating because it depicts graphically information desired by men of the types named above, most of whom are eager to acquire some proficiency in scoring milk.

8. The use of the chart has proved an asset in giving instruction in milk judging.

THE EFFECT OF ALFALFA HAY ON MILK FLAVOR

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Many workers have called attention to the fact that the feed a cow receives may have a pronounced effect on the flavor of the milk. Particular attention has been directed toward the dangers in using the succulent feeds. Peculiarly enough the injury to milk flavor that is caused by alfalfa hay seems in many cases to have escaped notice. Some investigators, in ascertaining the effects of various succulent feeds on milk flavor, have even used alfalfa hay in the check rations apparently in the belief the hay exerted no effect. On the other hand, Roadhouse, Regan and Mead (1), in 1926, recognized that alfalfa hay exerted an effect on milk flavor. They mentioned its effect was less pronounced than when the alfalfa was used as pasture or silage. Also Lucas (2), in 1929, said, "Alfalfa hay gives to milk a rather pronounced flavor but it is objected to by only a very few people."

Probably others both in research work and dairy practise have observed the effects of alfalfa hay on milk flavor but like Lucas considered these effects of little consequence or in a desire to avoid any criticism of alfalfa as a dairy feed deliberately omitted comment on their observations.

The interest of the authors in ascertaining the effect of alfalfa hay on milk flavor was prompted by two incidents. One of the better milk plants in Oklahoma had during 1931 encountered feed flavors, the source of which seemed to be alfalfa hay. Reluctance was felt in granting that this feed might cause as serious defects as were observed but a study of the question seemed justified.

At about the same time there was under way at this station a feeding trial in which mungbean hay was compared with alfalfa hay to determine their values in milk and butterfat production. Churnings were made of cream produced on each feed to determine the effect of the mungbeans on the quality of the butter. It was observed that the butter from the alfalfa hay rations carried a distinct feed flavor while that from mungbean hay failed to show the flavor and invariably received higher scores.

PROCEDURE

During the winter of 1933–34, we conducted a trial to determine the effect upon milk flavor of varying quantities of alfalfa hay fed at varying time intervals before milking. Twelve cows were used. They were kept in adjoining experimental stanchions except for five or six hours daily when they were kept in a dry lot. They were fed alfalfa hay and a concentrate

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mixture consisting, by weight, of 4 parts corn, 2 parts oats, 2 parts bran and 1 part cottonseed meal. Some of the cows received no succulent feeds, others received mangels and others darso silage.

The alfalfa hay used was grown locally and was fed from the bale. It was described as No. 1, leafy, fine, quite green and free from foreign matter. The interval before milking at which the hay was fed varied from one-half to seven hours. In this work involving the time interval four pounds of hay was always used as a standard quantity because it was about the proper amount for one feeding for the smaller cows weighing approximately 800 pounds. In the work wherein the quantity of hay was varied two, four and six pound quantities were fed at a uniform interval of one hour before milking.

The concentrate mixture was fed all cows just before the first cow in the string was milked. The mangels or silage was fed each cow immediately after she was milked.

The cows were milked twice daily by one man who seldom varied by as much as two minutes from his scheduled time for each cow. This close adherence to the milking schedule made it possible to devise a hay feeding schedule to provide the desired interval between feeding and milking.

The work on this problem was restricted entirely to the evening milkings which started at 3:45 P. M. As each cow was milked her milk was carried to the milk room. A sample was strained into a 10-ounce bottle. The bottle was capped and cooled down in tap water with frequent shaking to about 50° F. When all cows were finished at about 5:25 P. M., the samples were removed from the water and placed in the refrigerator at 40° F. till the next day at 8:00 A. M. when they were heated to about 95° F. and scored for flavor. The samples, identified only by number, were scored by six members of the staff. On the score card for milk 25 points are allowed for a perfect flavor.

RESULTS

The Interval Between Feeding and Milking

In this work, 12 samples from each cow were scored by six staff members which gave 72 observations on each cow or a total of 864 observations on the entire 12 cows. In table 1 are shown the average scores on all cows by individual judges and in the right-hand column the composite scores by all the judges.

The data in table 1 reveal considerable uniformity among the staff members in scoring the samples. Without exception the check samples—in the first line when the cows were fed hay after milking—scored highest. With four of the judges the milk collected after the 2-hour interval scored lowest. All judges' scores were quite consistently higher as the interval was prolonged beyond 2 hours.

TABLE 1

Scores by individual judges on all the cows' milk when alfalfa was fed

INTERVAL BEFORE MILKING	JUDGE'S INITIAL						ALL JUDGES
	F	Ku	W	M	Ke	R	
Fed after	21.9	22.2	22.3	21.9	21.9	21.4	21.9
½ hour	19.9	19.0	19.9	19.7	19.4	absent	19.6
1 hour	18.8	18.3	18.5	18.5	19.2	absent	18.7
2 hours	18.3	17.9	18.9	17.7	19.0	18.9	18.5
3 hours	19.3	18.8	19.8	19.0	19.5	18.9	19.2
4 hours	20.0	19.9	21.0	20.8	19.8	20.9	20.4
5 hours	21.2	21.0	22.0	21.6	20.3	21.6	21.3
6 hours	21.2	21.6	21.7	21.5	20.9	21.1	21.3
7 hours	21.1	21.9	21.8	21.5	20.6	21.0	21.3

The feeding of four pounds of hay one-half hour before milking decreased the scores from 21.9 to 19.6. The milk flavor was seriously affected. The 2-hour interval gave the lowest scores of all. This observation is in agreement with that of Roadhouse, Regan and Mead. Alfalfa hay exerts its worst effect if fed at this interval. Beyond the 2-hour interval the scores increased to 21.3 at the 5-hour interval and remained at that point for the 6- and 7-hour intervals.

The failure of the scores after the longer intervals to regain the level of the check samples is noticeable. In scoring these samples the judges were

TABLE 2

Scores by all judges on milks of individual Holsteins when alfalfa was fed

INTERVAL BEFORE MILKING	COW'S NUMBER				ALL FOUR HOLSTEINS
	1	2	3	4	
Fed after	22.6	22.1	22.3	21.6	22.2
½ hour	21.0	22.4	20.4	20.6	21.1
1 hour	21.2	21.0	19.0	18.8	20.0
2 hours	21.2	21.3	19.5	18.7	20.8
3 hours	20.5	22.2	19.2	21.0	20.7
4 hours	21.8	22.3	22.3	22.0	22.1
5 hours	21.0	21.8	21.2	22.6	21.7
6 hours	21.7	21.7	22.8	21.6	22.0
7 hours	21.8	21.7	22.4	21.8	21.9

extremely alert to any indication of a feed flavor. With some of the cows the effect of the alfalfa hay was entirely eliminated when fed four hours before milking. With other cows a slight off-flavor persisted in milk drawn even as long as seven hours after feeding. However this slight flavor was scarcely discernible; only a very small percentage of persons would detect it.

When the first scoring was done it became apparent that the large Holstein cows in the trial showed far less effect from the four pounds of hay than the smaller Jerseys. There were four purebred Holsteins and three purebred Jerseys. The remaining five cows in the trial consisted of two purebred Guernseys and three grades intermediate in body weight between the purebred Holsteins and Jerseys.

Table 2 shows the response of the Holsteins to the feeding of the hay. Table 3 gives corresponding data for the Jerseys.

TABLE 3

Scores by all judges on milks of individual Jerseys when alfalfa was fed

INTERVAL BEFORE MILKING	COW'S NUMBER			ALL THREE JERSEYS
	8	9	10	
Fed after	22.0	22.6	22.1	22.2
½ hour	18.2	18.2	17.8	18.1
1 hour	19.0	17.6	16.4	17.7
2 hours	17.2	16.0	16.8	16.7
3 hours	17.0	17.7	17.0	17.2
4 hours	18.0	20.3	18.2	18.8
5 hours	22.8	20.5	19.5	20.9
6 hours	21.2	21.5	20.8	21.2
7 hours	22.5	21.8	21.0	21.8

From tables 2 and 3 it is observed that the check samples from the Holsteins and the Jerseys scored exactly the same, 22.2 points. After the one-half hour interval the Holstein scores dropped only 1.1 points to 21.1. The Jerseys dropped 4.1 points to 18.1. Also the Holsteins showed the lowest score after the 1 hour interval while the Jerseys showed the lowest score after the 2 hour interval. With the Holsteins the milk obtained after the 4 hour interval or later was scored practically as high as the check samples, while the Jerseys still showed a slight effect on their milk even after the 7 hour interval. The flavor score of the milk from the Holsteins never dropped below 20.0. That of the Jerseys went as low as 16.7.

In the absence of a definite explanation of the difference in behavior between the two breeds, the conjecture is offered that this difference is

largely a matter of body weight. The four pounds of hay for the 1400-pound Holsteins is relatively less significant than for the 800-pound Jerseys. On the other hand, some additional factors may be involved. It is possible the Jersey milk because of its higher fat percentage carries more of the feed flavor than Holstein milk. Also there is the possibility the Jersey naturally produces milk with a higher flavor. Further attention is being devoted to this particular phase of the problem.

AERATION AND COOLING

In such a problem as this involving a study of milk flavor interest arises in the possibility of removing the flavor by aeration or cooling or both procedures. Some study has been directed along these lines. Cooling without aeration apparently was ineffective in removing the flavor. Aeration alone accomplished fully as much benefit as combined aeration and cooling, but even the aeration did not remove all the flavor.

It has been possible by aeration as well as by combined aeration and cooling to secure milk which scores about mid-way between the checks—those having no feed flavor—and the feed-flavored milk that was neither aerated nor cooled. In other words, the aeration removes about one-half the flavor imparted to milk by the hay.

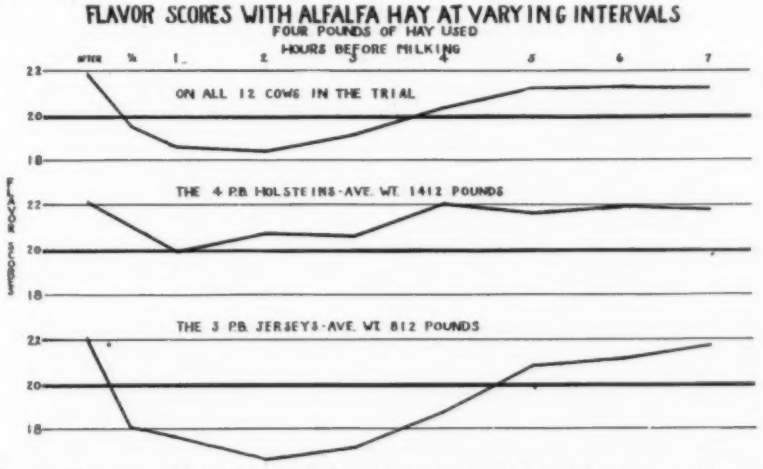

FIG. 1. EFFECT ON MILK FLAVOR OF FOUR POUNDS OF ALFALFA HAY FED AT VARYING INTERVALS BEFORE MILKING.

The results in all three of the tables given above are more readily apparent when the data are plotted as in figure 1. The top curve in this figure shows the scores on all 12 cows in the trial. It is plotted from the values in the right-hand column in table 1. The middle curve in the figure repre-

sents the data on the Holsteins from table 2 and the bottom curve on the Jerseys from table 3.

IDENTIFYING THE ALFALFA FLAVOR

We have been impressed with the fact the alfalfa hay imparts a characteristic flavor. With even slight experience one is able to distinguish the flavor from the silage or pasture flavor. Though the flavor is pronounced it was confused with "cowy" when first encountered at the start of the trial. As now recognized after considerable experience the milk carrying the alfalfa flavor gives a slightly sweet sensation when first taken into the mouth. Then a slight bitter or acrid taste appears and it persists for a few minutes even after the mouth is rinsed with warm water. There is some analogy between this flavor and that obtained from tasting good alfalfa hay. The odor as well as the taste is pronounced.

COMPARISON OF ALFALFA HAY AND SILAGE

In a trial in which darso silage instead of alfalfa hay was studied results were secured that were quite unexpected. The darso silage in 12-pound quantities was consistently less offensive in producing off-flavors in the milk than was the alfalfa hay in 4-pound quantities.

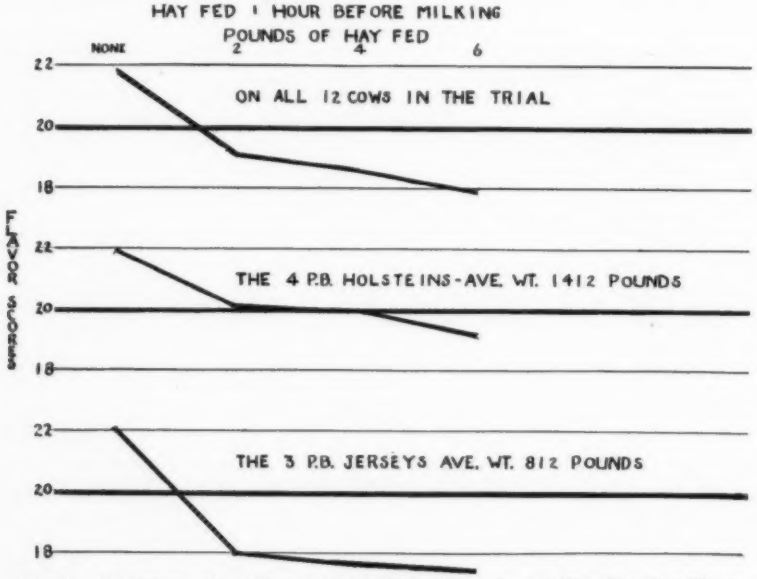

FIG. 2. EFFECT ON MILK FLAVOR OF VARYING AMOUNTS OF HAY FED ONE HOUR BEFORE MILKING.

The darso silage, while characteristic of this type of silage, was not of the quality that is found in good corn silage. It lacked the desired sharp odor of silage; it was also lighter and more fluffy. Possibly these reasons explain why it did not give as serious defects to the milk as did the hay.

It was of interest to observe that mangels in quantities of 20 to 35 pounds caused no injury to the milk flavor.

THE QUANTITY OF HAY FED

In this work some attention was devoted to the effects of different amounts of alfalfa hay on the milk flavor. Quantities consisting of two, four and six pounds were fed one hour before milking. Figure 2 gives the results secured in this phase of the trial.

From figure 2 it is observed that the 2-pound quantity of hay decreased the flavor score noticeably. The larger quantities occasioned further reduction in the scores but not in direct proportion to the amounts. Here, as in figure 1, it is observed that the Holsteins show far less effect than the Jerseys.

CONCLUSIONS

1. Alfalfa hay fed less than four hours before milking has a pronounced effect on milk flavor. This effect is observed even when the interval between feeding and milking is only one-half hour. The 2-hour interval causes the most serious flavor in the milk.

2. If the hay is fed as long as four hours before milking the flavor is entirely eliminated with some cows. With other cows it is so reduced as to be scarcely discernible.

3. The same precautions should be used in feeding alfalfa hay as are recommended for such feeds as silage; it should be fed after milking or at a sufficient interval before so that its possible effect on the milk flavor will have been eliminated.

4. The intensity of the alfalfa flavor in the milk increases as the amount of hay fed the cow is increased.

5. The effect of the hay on the milk flavor is considerably less serious with Holsteins than with Jerseys.

6. Aeration of the milk removes some of the flavor but does not entirely eliminate it. Cooling seems to be ineffective.

7. The effect of alfalfa hay is far more serious than that of darso silage.

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THE PREPARATION AND NUTRITIVE VALUE OF A.I.V. SILAGE FOR DAIRY COWS*

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LITERATURE REVIEW

During the last few years a number of attempts have been made to perfect improved methods of ensiling fodder. For the most part these efforts have been directed toward the prevention of losses of nutrients, the prevention of the formation of objectionable substances in silage and toward the ensiling of crops which because of high protein content or other feature have not been customarily ensiled. Methods have been suggested by Hölken (1) and others for the application of heat from the outside or for the regulation of the amount of heat developing within the silage. Gorini (2) has obtained good results with this latter method. The object of heating is to produce a temperature favorable for the development of lactic acid bacteria but unfavorable for objectionable forms.

Considerable work has been directed toward overcoming the difficulties involved in ensiling legumes. In general protein decomposition is the greatest obstacle to such a practice, involving as it does, a loss of valuable nutrients and the production of compounds such as ammonia which are undesirable from the standpoint of odor and palatability. Schieblich (3), Shutt (4), and Wright and Shaw (5) have reported favorably on the practice of wilting legume crops before ensiling. Neal and Becker (6), Ragsdale and Turner (7), and Westover (8) were able to prepare good legume silage without wilting; but the loss of crude protein as reported by Neal and Becker was 34 per cent. Various substances have been added to legume crops during the process of ensiling in order to hasten lactic acid production and so to inhibit proteolytic enzymes of both plant and bacterial origin. Kuchler and Wachter (9), Shutt (4), and Schmidt (10) have tested the efficacy of these methods.

Rapid formation of lactic acid, although desirable from the standpoint of protein preservation, involves necessarily the destruction of a part of the carbohydrate content of the silage. In order to prevent this as well as to insure complete preservation of proteins Virtanen (11–17), has worked

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out a method of silage preservation entailing the addition of acids or acid compounds to the green fodder. Various mixtures of HCl and H_2SO_4 may be used, and the mineral acids may be replaced by other compounds such as lactic acid, $NaHSO_4$, $S_2O_3Cl_4$, $SOCl_2$, SO_2Cl_2, and $ClHSO_3$. The acid is sprinkled on the fodder in sufficient quantity to produce a pH of from 3.6 to 4.0. According to Virtanen, fodder with a pH of less than 3.0 is unsuitable for consumption, and if the pH is above 4.0 proteolysis and deamination take place with consequent loss of feeding value. It is claimed for this method that it checks plant cell respiration and bacterial activity, inhibits proteolysis, preserves soluble carbohydrates, carotene, and vitamin C, and prevents losses due to poor weather conditions at the time of harvesting the crop. Virtanen states that silage prepared according to this method contains from 0.52 to 0.80 per cent of lactic acid on the fresh basis and from 0.28 to 0.38 per cent acetic acid. When clover silage was preserved at a pH of 3.7, there was no increase in water soluble nitrogen or ammonia nitrogen; however, if the pH was 4.1, the ammonia nitrogen increased to 12 per cent of the total nitrogen, and the water soluble nitrogen increased from 26 to 44 per cent of the total nitrogen. There was no appreciable loss of carotene and only 50 per cent destruction of vitamin C, which, as is well-known, is completely destroyed in the process of making hay.

Legume silage prepared in this way, because of its relatively high protein content, has been substituted with good results for part or all of the concentrates of a winter ration. The possibility of thus eliminating high-priced concentrates is of considerable importance. According to Virtanen, cows receiving such a winter ration maintained their butterfat production at summer levels and the butter made from their milk simulated summer butter, having a higher carotene content and a higher iodine number than ordinary winter butter. The cows manifested no ill effects due to the acid, either from the standpoint of general condition or from the standpoint of the ash content of the bones and other organs. In order to compensate for the mineral acid added to the forage, chalk should always be included in the ration.

This method of preserving fodder has been named the A.I.V. method after Professor A. I. Virtanen, its sponsor and originator. It has found widespread use in Finland and Sweden and is protected by patents in those countries and in the United States, Canada, and Great Britain.

Sjöberg and Köhler (18) investigated the A.I.V. method of preserving cabbage leaves with HCl. The lowest pH attained was 3.8, and they found that at this acidity there was a very slight loss of carbohydrate and of protein, but no appreciable amounts of ammonia or organic acids were produced. A pH of 4.5 was sufficiently low to suppress completely the formation of butyric acid.

Other methods of preserving fodder by the addition of acid have been described by Gerlach (19) and Ruschmann (20), but neither of these has come into such general use as has Virtanen's method.

The experiments described herewith were undertaken for the purpose of investigating various aspects of the A.I.V. method, particularly with reference to the preservation of protein and carotene, the presence or absence of fermentative changes, the effect upon cows of feeding the silage as part of a winter ration, and the adaptability of the method to conditions of silage making in this country.

PART I. PREPARATION AND COMPOSITION OF SILAGE

Filling of Silo

Three lots of silage were put up, designated as Alfalfa I, Alfalfa II, and Soy Beans, and consisting respectively of 11.2 tons, 17.1 tons, and 9.7 tons. The amounts of acid to be added were determined according to the procedure described by Virtanen. Representative samples were taken from the field a day or two before filling the silo and chopped up. Each of three 200 gram samples was then ground in a mortar with about 100 cc. of distilled water. To the three samples were added respectively 6, 12, and 18 cc. of 2 N acid. The plant material and acid were thoroughly mixed in the mortar and then transferred with as little water as possible to glass jars in which the acidified plant material was pressed down by means of a bottle filled with water and allowed to stand for 24 hours. After this length of time each sample was thoroughly mixed in a large evaporating dish, part of the juice was pressed out, and the pH of the liquid was determined by either the quinhydrone or the glass electrode. If none of the three samples gave exactly the desired pH, the pH values were plotted against amounts of acid added; and from the curve, which is practically a straight line, the amount of acid necessary to produce a pH of 3.5 was determined. This amount was increased by 10 per cent to provide a margin for errors in sampling. As may be seen from table 1, the amount of acid required

TABLE 1

Quantity and cost of acid used per ton of silage

ITEMS	ALFALFA 1	ALFALFA 2	SOY BEANS
2 N acid, HCl–H_2SO_4, l.	90	120	100
Commercial HCl, 18° Bé, lbs.	30.5	48.2	40.2
" H_2SO_4, 65° Bé, lbs.	8.6	9.7	7.9
$\frac{HCl}{H_2SO_4}$, mols	2.75	3.92	3.92
Cost of HCl, cts.*	48.8	77.2	64.3
" " H_2SO_4, cts.*	13.7	15.5	12.6
Total cost of acid, cts.*	62.5	92.7	76.9

* Prices as quoted in *Oil, Paint and Drug Reporter*, July 30, 1934.

varied considerably, this variation depending upon the state of the crop when ensiled. The second lot of alfalfa was more mature than the first, which probably explains the higher acid requirement.

The addition of the acid was accomplished by various methods. In the case of the first lot of alfalfa, the acid was hoisted to the top of the silo in 20 liter bottles and siphoned out, or forced out with a small air pump, through a sprinkler onto the alfalfa which was run into the silo through a cutter in the usual way. In the case of the second lot of alfalfa, due to the proximity of the silo to an elevated driveway, it was possible to mix the acid at the top of the silo and siphon it into the silo. The alfalfa was not cut up and blown into the silo but was pitched in from the top. These conditions could not ordinarily be duplicated except by the use of a pit silo such as is employed in Finland. This lot of alfalfa and the soy beans were put up under the supervision of Mr. G. L. Rosenquist of the Valio Laboratories, Helsinki, Finland. The acid applied to the soy beans, which were cut up as was the first lot of alfalfa, was pumped to the top of the silo through a garden hose by means of an electrically-driven centrifugal pump. The stream of acid was delivered to the mouth of the blower pipe so that it was mixed thoroughly with the cut fodder emerging from the pipe. The proportions of hydrochloric and sulfuric acids were varied as indicated in table 1. From the standpoint of economy it is best to use as high a proportion of H_2SO_4 as possible; but from the standpoint of feeding, it is not desirable to have too great a quantity of sulfates in the silage.

The most important factor in determining the cost, however, is the quantity of acid required. The lowest figure in table 1, 62.5 cents per ton, corresponds to an application of 90 liters. It is probable that in many cases, *e.g.*, with more succulent materials, the quantity of acid and hence the cost could be reduced. The cost would also vary with the quantity of acid purchased in a single order. The prices used in calculating the figures given in table 1 were those quoted for lots of 25 carboys or more, *viz.*, $1.60 per 100 lbs. for both commercial muriatic acid and commercial sulfuric acid.

The first lot of alfalfa and the soy beans were covered with layers of corn silage about 2 feet thick. The second lot of alfalfa was covered as recommended by Virtanen. Double portions of acid were added to the last 1000 pounds of fodder, and to the last portion of acid was added about 100 cc. of a mustard oil emulsion in order to prevent mold growth. The surface of the silage was covered with tar paper on top of which was placed a 4 inch layer of moistened shavings and an 18 inch layer of soil.

The silo in which the second lot of alfalfa was ensiled was provided with a drain so that the liquid draining to the bottom could be removed, collected, and analyzed.

The fresh plant material and the silage were analyzed for dry matter, total nitrogen, total water soluble nitrogen, amino nitrogen, ammonia nitro-

gen and carotene. To a limited extent the bacterial population and the organic acid content of the silage were also investigated.

Composition of Silage

The losses from the second lot of alfalfa silage by drainage of juice are set forth in table 2. It may readily be seen that the losses of dry matter

TABLE 2
*Losses from alfalfa silage 2 by drainage of juice**

	DRY MATTER	NITROGEN	2 N ACID
	kg.	*kg.*	*liters*
Silage	5030	153	2075
Lost in drainage juice	89.2	1.40	237
Percentage loss	1.77	0.91	11.4

* Total volume of drainage juice was 1,476 liters.

and of nitrogen in this way were negligible. The loss of acid, expressed in liters of 2 N acid, corresponded quite closely to the 10 per cent excess of acid added to allow for this loss. As might be expected, the first juice to drain out was the most acidic, having a pH of 1.37; whereas the last to drain out, 13 days after the filling of the silo, had a pH of 3.73.

Tables 3 and 4 show some of the changes occurring in plant tissue when preserved as A.I.V. silage over a period of 4½ to 5½ months. It was of course impossible to obtain a single sample representative of the contents of the silo as a whole and this fact together with the possibility of drainage downward of acid and of soluble nitrogen compounds probably accounts for the variable results obtained on different samples from the same silo. The high content of water soluble nitrogen would increase the possibility of downward movement of nitrogen. In this connection it should be mentioned that the low pH value of 2.1 recorded for the soy beans and the high figure for ammonia nitrogen recorded for the second lot of alfalfa were both obtained on samples taken from near the bottom of the silo. The pH values, except that mentioned above correspond fairly well with Virtanen's recommendations. The nitrogen analyses show marked increase in water soluble nitrogen and amino nitrogen, but no significant amounts of ammonia were found. Even in the above mentioned case, the second lot alfalfa sample, taken from near the bottom of the silo, contained only 0.1 per cent of ammonia nitrogen. The marked increases in water soluble nitrogen are not in accord with the results obtained by Virtanen who found, as stated above, that when the pH was 3.7 there was no increase in water soluble nitrogen. The pH was higher than this in the second lot of alfalfa, which fact would explain the difference in this case, but this explanation does not apply to the other two silages. The increases in water soluble nitrogen

TABLE 3
Analyses of fresh plant material and silage

	pH	DRY MATTER	TOTAL NITROGEN DRY BASIS	WATER SOLUBLE NITROGEN, DRY BASIS	AMINO NITROGEN, DRY BASIS	AMMONIA NITROGEN, DRY BASIS	CAROTENE, DRY BASIS
		%	%	%	%	%	*Micrograms per gm.*
Alfalfa 1							
Plant material		34.6	2.65	0.818	0.386	0.0289	129**
Silage	3.3	29.8	2.51	1.128	0.462	0.0288	139
Alfalfa 2							
Plant material		32.3	3.04	0.446	0.190	0.0065	57**
Silage*	3.9–4.2	30.8	2.83	1.149	0.457	0.0359	78
		38.3	3.25	1.257	0.677	0.1016	83
Soy Beans							
Plant material		27.2	2.57	0.514	0.254	0.0159	88**
		23.1	3.03	1.064	0.459	0.0177	131
Silage*	2.1–3.9	25.1	3.11	1.085	0.499	0.0519	217

* Analyses of 2–4 samples from various depths in silo.
** These figures are low because of incomplete extraction.

TABLE 4
Nitrogen distribution in fresh plant material and silage

	TOTAL NITROGEN	WATER SOLUBLE NITROGEN	AMINO NITROGEN	AMMONIA NITROGEN
	%	%	%	%
Alfalfa 1				
Plant material	100	30.9	14.6	1.09
Silage	100	44.9	18.4	1.15
Alfalfa 2				
Plant material	100	14.7	6.25	0.21
Silage	100	35.4–44.4	14.1–23.8	1.26–3.12
Soy Beans				
Plant material	100	20.0	9.88	0.62
Silage	100	34.9–35.1	14.7–16.5	0.58–1.67

TABLE 5
Data on fermentation in silage

	ORGANISMS PER GM. OF SILAGE	ORGANIC ACIDS, FRESH BASIS			
		Volatile, as acetic	Non-volatile as lactic	Lactic	Total, as lactic
	millions	%	%	%	%
Alfalfa 1					
After 2 weeks	8	.....	.....	.....	1.10
Alfalfa 2					
After 5 months	1.2	0.70	1.72	0.946	2.76

and amino nitrogen are probably not of very serious consequence since increases in these forms of nitrogen do not necessarily mean decreased feeding value; whereas an increase in ammonia nitrogen detracts from both palatability and feeding value, since ammonia is unavailable to the animal.

Increases in the carotene content of the silage as compared with the fresh plant material were obtained consistently, and the increases were quite significant in most cases. These increases were very puzzling until after the experiment was completed—when it was found that treatment of fresh plant tissue with acid for 24 hours increased the carotene values markedly. It appears then that these apparent increases may be due merely to the action of the acid in increasing the extractability of the carotene.

The data on fermentation in the silage (table 5), though fragmentary, indicate that bacterial changes are by no means entirely suppressed. The bacterial count of 8 million organisms per gram obtained on the first lot of alfalfa two weeks after ensiling, while not an exceptionally high count, indi-

cates considerable bacterial growth. The later count made on a sample from the second lot of alfalfa 5 months after ensiling shows that a considerable number of microorganisms persisted even after this length of time. The amounts of volatile and non-volatile acids are approximately those found in ordinary silage. However, only the volatile and lactic acids should be attributed to fermentation process. The difference between non-volatile acid and lactic acid (0.67%) probably represents plant acids present in the original green alfalfa. The 0.946 per cent of lactic acid is somewhat in excess of the 0.52 to 0.80 per cent reported by Virtanen. Since the pH of the second lot of alfalfa silage ranged from 3.9 to 4.2, these results perhaps should not be compared with those of Sjöberg and Köhler who found that no appreciable quantities of organic acids were formed in cabbage preserved at a pH of 3.8. Factors other than acidity, as for instance the chemical and physical characteristics of the plant material, probably account for this great difference in organic acid content of silages differing only slightly in pH. The relatively high organic acid content of this lot of silage might well be expected on the basis of the rather large amount of bacterial activity indicated by the bacterial counts.

PART II. FEEDING EXPERIMENT

Rations and Feeding of Animals

Two lots of five dairy cows each were used in a double reversal feeding trial which extended over a six week fore-period, a seven week A.I.V. period, and a seven week after-period. During the control periods Lot I (three Holsteins and two Guernseys) was fed a grain mixture consisting of equal parts of corn and oats, plus alfalfa hay and corn silage, while Lot II (two Guernseys, two Jerseys and one Brown Swiss), received a grain mixture, 40 per cent corn, 25 per cent oats, 20 per cent gluten meal, and 15 per cent linseed meal, plus timothy hay and corn silage. A pound of grain was fed for every 3½ pounds of milk produced and 1 pound of hay and 3 pounds of corn silage per 100 pounds of live weight. In the A.I.V. silage period all cows received a 50 : 50 corn-oats mixture, half the former amount of alfalfa or timothy hay, no corn silage and A.I.V. alfalfa silage *ad libitum*. Approximately one per cent of iodized salt was added to the grain mixtures. In order to compensate partially for the high acid intake on the A.I.V. ration, 4 ounces of $CaCO_3$ were fed daily in the grain mixture of each cow. Accurate records were kept of the amount of feed consumed, weight of cows, and milk and butterfat produced.

Lots I and II were not set up for the purpose of comparing one with the other but were conveniently divided in that manner because of the regular feeding schedule in use with the university herd. The cows in Lot I had been receiving alfalfa hay as their dry roughage prior to the beginning of the experiment, while the cows in Lot II had been getting timothy

hay. The cows were stall fed and had access to drinking water at all times.

At the beginning of the A.I.V. period the cows did not relish this higher acid taste, but, by increasing gradually the amount of A.I.V. silage fed, at the end of two weeks they were consuming from 30 to 50 pounds daily. On an average the cows consumed 50 per cent more A.I.V. silage than corn silage on the dry matter basis due to the fact that they were receiving only half as much hay. After the first two weeks of feeding none of the cows indicated an apparent dislike for this new type of silage. However, the feces of the cows on A.I.V. silage became very soft simulating the droppings of animals on pasture, a condition which was particularly noticeable in the group receiving timothy hay.

Effect on Milk Production

Butterfat and milk production records indicated that the feeding of A.I.V. silage did not appear to have any unusual influence on milk production as the lactation curves from the two groups of cows appear to be approximately normal (Figure 1). However there were some irregularities

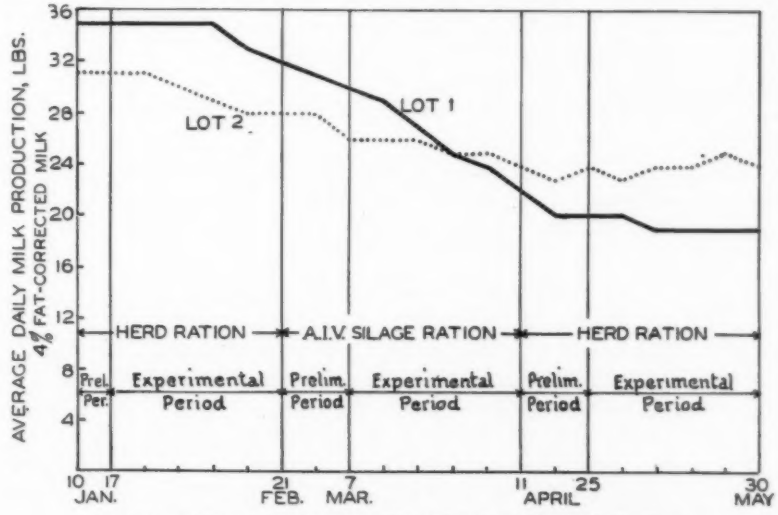

FIG. 1. EFFECT OF A.I.V. ALFALFA SILAGE ON MILK PRODUCTION

in the production curves during A.I.V. feeding which were probably due to the low intake of the silage during the adjustment period. A seven-week trial does not present enough data to be conclusive as to its effect on milk production. This can only be answered by experiment of longer duration, such as an entire lactation period.

Effect on the Carotene and Vitamin A Control of Butterfat

Individual butter samples were prepared from a composite 24 hour milk sample of cows of the same breed at definite intervals during the three periods. Both carotene and vitamin A content of the butter samples were determined spectroscopically by methods previously described (21, 22). These analyses indicated a definite increase in both carotene and vitamin A with the inclusion of A.I.V. silage in the ration (Figure 2). In most cases

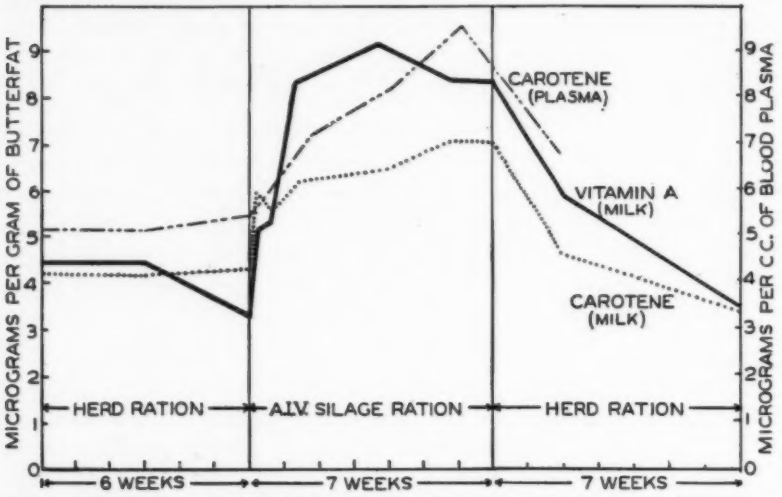

FIG. 2. INFLUENCE OF A.I.V. SILAGE ON THE CAROTENE AND VITAMIN A CONTENT OF MILK AND THE CAROTENE CONTENT OF BLOOD PLASMA (AVERAGE FROM TEN COWS OF VARIOUS BREEDS)

the maximum content of both of these components was reached in 4 weeks. This was a somewhat slower increase than we have found with cows on pasture (21), but it is probably due to the low intake of silage during the first two weeks of feeding. An average of the carotene for ten cows during the control period gave a value of 4.2 micrograms of carotene per gram of butterfat with an increase to 6.8 micrograms per gram during the A.I.V. period. Vitamin A analyses showed an average value of 4.0 micrograms per gram of butterfat for the control period and a definite rise to 9.0 micrograms per gram in the A.I.V. period. Both carotene and vitamin A indicated a definite drop within two weeks after the A.I.V. silage was discontinued, and were definitely at the control period level after seven weeks. The changes in carotene and vitamin A are shown more clearly by comparing the differences within the same breed. It was found in this study as well as in previous work (21) that the Holstein milk is high in vitamin A and low in carotene, Guernsey milk is low in vitamin A and high in caro-

tene, and milks from Jerseys and Brown Swiss occupy intermediate positions. Therefore when the results were averaged the breed differences in these components tended to neutralize the changes.

Although the feeding of A.I.V. silage gave a definite rise in both carotene and vitamin A, the values obtained were not so high as those obtained by a previous study with cows on pasture (21). A.I.V. silage gave a value of 9.5 micrograms per gram for the carotene of Guernsey butterfat, while the same cows on pasture yielded a butterfat with 17.0 micrograms of carotene per gram. The vitamin A of the Holstein butterfat was increased from 10 to 15 micrograms per gram by pasture feeding. The other breeds showed similar differences between A.I.V. and pasture feeding in both carotene and vitamin A (Table 6). These differences were no doubt due

TABLE 6

Effect of Winter ration, A.I.V. silage ration, and pasture on the carotene and vitamin A content of butterfat

(In micrograms per gram of butterfat)

BREED	CAROTENE			VITAMIN A		
	Winter ration 180 days	A.I.V. alfalfa silage 49 days	Pasture 20 days*	Winter ration 180 days	A.I.V. alfalfa silage 49 days	Pasture 20 days*.
Guernsey	6.0	9.5	17.0	2.9	8.5	8.7
Holstein	3.5	5.4	6.6	4.0	10.0	15.0
Jersey	3.7	7.1	10.7	3.1	7.7	10.2
Brown Swiss	3.4	5.0	9.8	5.8	9.7	12.6
Ave.	4.2	6.8	11.0	4.0	9.0	11.6

* These results were taken from analyses of butters from various breeds within the herd prior to this experiment.

in part to a lower consumption of A.I.V. silage than pasture, thus resulting in a lower carotene intake. The lower consumption of A.I.V. silage as compared to pasture may be partly attributed to the fact that the cows were receiving hay with A.I.V. silage, while the pasture cows received no dry roughage.

Studies on the carotene content of the blood plasma paralleled the increases in the butter (Figure 2). The average carotene content of the plasma on a normal ration was 5.2 micrograms per cc. with a definite increase to 9.6 micrograms per cc. on the A.I.V. ration (Figure 2).

Balance studies on the total carotene intake in the feed and the total carotene and vitamin A output in the milk indicated that approximately doubling the carotene consumption due to A.I.V. feeding also doubled the total vitamin A content of the milk (Table 7). Expressed in terms of total

TABLE 7

Carotene balance of cows on herd and A.I.V. ration
(Average carotene intake and output per animal)

	LOT I		LOT II	
	Herd ration	A.I.V. ration	Herd ration	A.I.V. ration
Carotene in 10 lbs. grain, mg.*	10	9	18	8
Carotene in roughage, mg.	347	552	180	502
Total carotene fed daily, mg.†	357	561	298	510
Total vitamin A output daily‡	6,644	11,389	5,894	10,785

* Carotene content per pound was as follows: grain mixture of Lot I 0.091 mg.; grain mixture of Lot II 1.8 mg.; alfalfa hay 11.5 mg.; timothy hay 2.1 mg.; corn silage 5.9 mg.; and A.I.V. alfalfa silage 13 mg.

† 1 mg. of carotene is equivalent to 1000 International rat units of vitamin A.

‡ International rat units of vitamin A.

international units of vitamin A produced on an average by each cow, our figures showed that during the control period the cows secreted in the milk on an average 6,269 units of vitamin A per day and on the A.I.V. ration the figure was raised to 11,087 units. Calculations of the intake and output of carotene in the same units showed that the animals received an enormous excess of carotene over the amount secreted. On both rations only 2 per cent of the ingested carotene of the feed appeared in the butterfat. Ordinarily an increase in the carotene ingested decreases the percentage absorbed (21), but with the addition of A.I.V. silage to the ration this did not appear to be the case. There is evidently a tremendous amount of the ingested carotene unabsorbed, or some of it is destroyed by certain acids and microorganisms (24, 25). Furthermore all the carotene absorbed is not secreted in the butter but may be either stored in the tissues, utilized directly by the tissues, or converted into vitamin A. A study of the actual percentage of ingested carotene utilized by the cow would no doubt yield interesting results.

Nutritive Value of A.I.V. Milk

In connection with a larger program on the nutritive value of milk produced at different seasons of the year (23), the nutritive value of A.I.V. milk was determined by feeding experiments with rats. The A.I.V. milk, fortified with Fe, Cu, and Mn, was fed *ad libitum* for six weeks to 6 male rats having an initial weight of 50 grams each. The rats receiving A.I.V. milk made an average gain in six weeks of 144 grams, while controls fed winter milk, fortified with the same elements, gained only 102 grams. This increase in growth was probably not due to the greater supply of vitamin A in the A.I.V. milk (37 units per day) since the controls received 17 rat units

per day, an intake which is many times the amount needed to meet the vitamin A requirement. The reasons for the superior growth of rats on the A.I.V. milk cannot be explained at the present time. Further research is in progress to throw some light on this question.

Effect on the Composition of the Blood and Urine

Five blood samples were analyzed at definite intervals during the course of the experiment in order to determine the effect of the high acid intake on their composition. The blood was collected under paraffin oil using sodium oxalate as an anti-coagulant, centrifuged immediately and the pH determined at 25° C. with a quinhydrone electrode in a closed chamber (26). The alkaline reserve was determined with a Van Slyke manometric blood gas apparatus (27). The alkaline reserve of the blood was lowered on an average from 66.3 cc. per cent for the control period to 56.7 cc. per cent

TABLE 8

Effects of feeding A.I.V. silage on composition of blood and urine

BLOOD PLASMA	FORE-PERIOD	A.I.V. PERIOD			AFTER-PERIOD
	3 week average	2nd week	4th week	6th week	2nd week
Alkaline reserve, cc. %					
Lot I—Alfalfa-fed cows	63.8	61.2	61.7	59.5	71.0
Lot II—Timothy-fed cows	63.8	53.9	53.1	53.8	66.1
pH					
Lot I	7.45	7.40	7.42	7.43	7.48
Lot II	7.45	7.37	7.40	7.40	7.45
URINE	**2 days before**	**3rd week**		**6th week**	**2nd week**
Fixed CO_2, cc. %					
Lot I	519.4	2.6		.....	405.8
Lot II	31.8	4.0		.....	79.2
pH					
Lot I	8.08	5.76		6.13	8.01
Lot II	6.97	6.08		6.15	7.20
NH_3-N, gm./100 cc.					
Lot I	.0006	.081		.120	.009
Lot II	.019	.133		.131	.034
Total-N, gm./100 cc.					
Lot I	.75	.84		.89	1.47
Lot II	1.68	1.12		1.16	1.85
Percentage of total N as NH_3N					
Lot I	.08	9.6		13.5	.61
Lot II	1.13	11.9		11.3	1.84

for the A.I.V. period (Table 8). These reductions due to A.I.V. feeding are not great enough to bring about a condition of acidosis. The slight

lowering of the pH of the blood is insignificant (Table 8). There was a prompt return of the alkaline reserve and pH to the former levels within two weeks after the feeding of A.I.V. silage was discontinued.

Four urine samples from each cow were analyzed. pH was measured with the quinhydrone electrode, fixed CO_2 with the Van Slyke manometric blood gas apparatus (27), ammonia nitrogen by the Folin aeration method as modified by Steel (28), and total nitrogen by the Kjeldahl method. The fixed CO_2 content of the urine indicated a drastic reduction from the control period to the A.I.V. period, the average being 276 cc. per cent for the former and 3 cc. per cent for the latter. The pH was lowered on an average from 7.5 to 5.9 and the ratio of the ammonia nitrogen to the total nitrogen was increased from 0.6 to 11.0 per cent (Table 8). The values for fixed CO_2, pH, and ammonia returned to normal after the cows were on the regular ration two weeks. These data show that more than an average quantity of acid was consumed by the cows and in part was neutralized by means which apparently prevented any deleterious effects on the health of the animals. However, it must be kept in mind that our observations extend over only a relatively short feeding period and it cannot be assumed that these results are sufficient to allow us to draw definite conclusions as to the ultimate effect of continuous feeding of A.I.V. silage.

SUMMARY

The preparation by the A.I.V. method of two lots of alfalfa and one of soy bean silage, consisting respectively of 10, 17, and 11 tons, is described.

The data show that the loss of nitrogen and of dry matter in the drainage juice amounted to 0.9 and 1.8 per cent respectively.

There were marked changes in the distribution of nitrogen in the silage. Based on the total nitrogen, the water soluble nitrogen increased in the two lots of alfalfa silage from 31 to 45 per cent and from 15 to 40 per cent respectively, and from 20 to 35 per cent in the soy bean silage. Increases in amino nitrogen paralleled those of the water soluble nitrogen. Ammonia nitrogen also increased, but the absolute quantity was small.

There was apparently no loss of carotene.

Counts of the number of bacteria and determination of volatile acids and lactic acid showed that some fermentation took place in spite of the low pH of the silage.

A double reversal feeding trial was conducted consisting of a six week fore-period, a seven week A.I.V. silage period, and a seven week after-period with ten dairy cows of various breeds in two groups of five each.

No unusual changes were noted in milk production due to the feeding of A.I.V. silage.

Spectroscopic analyses of the butterfat for carotene and vitamin A indicated a definite increase in these components with silage feeding. During

the control period an average value of 4.2 micrograms of carotene per gram of butterfat was obtained and in the A.I.V. period the figure rose to 6.8. Vitamin A analyses showed an average value of 4.0 micrograms per gram of butterfat for the control period and a marked rise to 9.0 in the A.I.V. period. However, the values for carotene and vitamin A during the A.I.V. feeding were not as high as those previously found for pasture feeding. Increases in the carotene content of the blood plasma paralleled those in the butterfat. Balance studies on the carotene intake in the feed and output in the milk showed a corresponding increase in both of these factors with the inclusion of A.I.V. silage in the ration.

Blood and urine analyses indicated that the high acid intake of the cows was neutralized by means which prevented any noticeable deleterious effects on the animals.

ADDENDUM

After this manuscript was prepared the paper by Watson, *et al.* (Biochem. J. 28, 1076, 1934) giving the effect of feeding grass ensiled by the A.I.V. method on the carotene and vitamin A content of butter appeared. These authors report a marked increase in both carotene and vitamin A as a result of feeding this fodder.

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American Dairy Science Association Announcements

PROBLEMS IN TEACHING AT JUNE MEETING

President C. L. Roadhouse has indicated that the time will be ripe next June for another special session for the members of the Association who are interested in a discussion of teaching problems and methods. It is urged that the Heads of Departments of dairying and dairy husbandry in the various colleges who have inaugurated new curricula or innovations or modifications of such teaching practices arrange to have these presented and discussed at the June meeting of the Association. Inasmuch as there is no official Section of Dairy Instruction with duly elected officers, the Chairman of the Program Committee will be glad to arrange for temporary officers and the Program Committee will arrange a special program for such a temporary section if sufficient titles are sent in.

L. S. PALMER, *Chairman Program Committee*

REPORT OF COMMITTEE ON EVALUATION OF PROVEN SIRES

The correct evaluation of a sire's transmitting ability requires a correct record of what his daughters have produced under standard conditions and somewhat less important, of what the dams of those daughters have produced under equivalent conditions.

The committee feels that more research on the subject and more experience in the actual use of these indexes will be required to justify official choice of any index. For this reason the committee is unwilling to recommend any specific index as correct and best under all conditions.

While practical experience in the use of these indexes is being increased, it is important that the fundamental data on which they rest should be presented for practical use in standardized averages such that those interested in the use of an index could easily compute any of the more promising indexes he may choose.

The committee, therefore, recommends to the Breeds Relations Committee that they ask the Breeds Association to publish the following information for all bulls with five or more tested daughters out of tested dams or with eight or more tested daughters even though less than five of these are out of tested dams:

1. The total number of registered daughters and the number of registered daughters more than four years of age.
2. The average production of all tested daughters. (By "production" is meant yield of milk, per cent fat, and total fat, corrected to a standard age and to a standard classification for number of times milked and to a standard length of lactation, preferably 305 days.)
3. The average production of all daughters out of tested dams.
4. The average production of those dams.
5. The number of daughters which exceed their dams.
6. The range or other measure of variation in the daughters' production.

Committee: F. W. ATKESON, Idaho; J. L. LUSH, Iowa; C. W. TURNER, Missouri; R. R. GRAVES, U. S. D. A.; H. O. HENDERSON, W. Virginia, Chairman.

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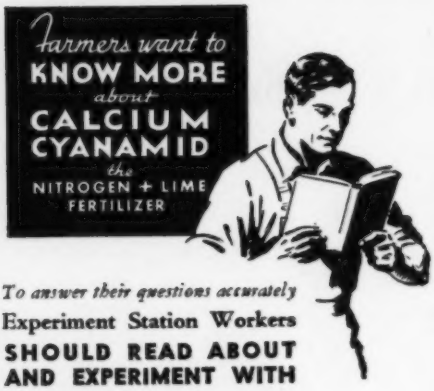

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*Facts you should know about

GLASS-LINED PASTEURIZERS

FACT 3

III. ***Insulation***—A standard feature with Pfaudler Pasteurizers is the use of pure compressed Corkboard insulation—cork that is cellular in structure. Thus loss of heat is retarded by millions of dead air cells, reducing both heating and cooling costs.

Temperatures are maintained evenly, meeting Health Department requirements in bacteria destruction and also protecting natural flavor and texture of the milk. In the new Pfaudler "Lo-Vat" Pasteurizer, the new one piece non-staining cover is also insulated. This cover fits snugly over the vat flange cutting temperature losses even more.

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THE PFAUDLER CO.

1515 Gas & Electric Bldg., Rochester, N. Y.

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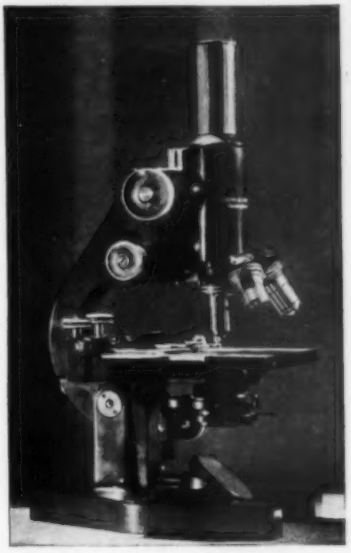

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Many dairies are making milk examinations more *quickly* and more *accurately* by using the Breed and other officially approved methods which involve the counting of bacteria rather than colonies.

The Bausch & Lomb H A Microscope is *ideally adapted to this type of work.* An extra large stage and smoothly operating mechanical stage aid in the systematic examination of large slides. Accessories for the various counting methods are available.

Stone Colony Counter

For convenience in counting colonies of bacteria, Bausch & Lomb offer a Stone Colony Counter mounted on an adjustable stand.

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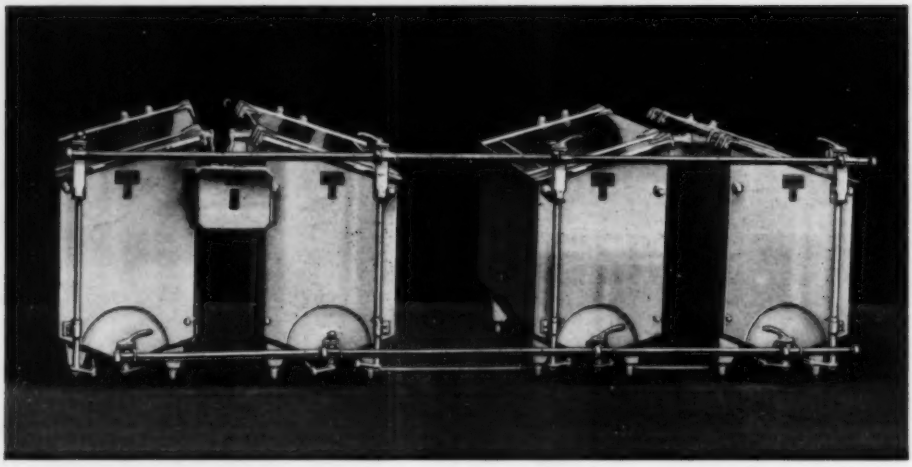

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AUTOMATIC SPRAY VAT HOLDER

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YOU already know the CHERRY-BURRELL SERIES "CC" SPRAY PASTEURIZER as America's outstanding batch pasteurizer . . . a saver of time and manual operations . . . an unfailing guardian of cream line and flavor and health . . . simple, accurate and positive . . . low in operating and maintenance costs.

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